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**GROWTH HORMONE DISTURBANCES
IN ADOLESCENTS
WITH TYPE 1 (INSULIN-DEPENDENT)
DIABETES MELLITUS**

Subtitle Differentiating the GH signal

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**THESIS PRESENTED FOR
MEDICINAE DOCTOR
UNIVERSITY OF GLASGOW**

**Research conducted at Department of Paediatrics, John Radcliffe
Hospital, Headington, Oxford during 1989-92**

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Abstract

This Thesis provides evidence that raised plasma growth hormone (GH) levels play an intrinsic metabolic role in Type 1 diabetes. Physiological and functional aspects of GH pulsatility has been examined under standardized conditions.

Diabetic subjects have prolonged GH clearance characteristics: Bolus GH half-life ($t_{1/2}$) measurements and distribution volume (DV) are increased (mean \pm SEM $t_{1/2}$ 13.6 ± 1.1 vs control 8.9 ± 0.54 mins, $p < 0.05$; DV 6.1 ± 0.4 vs control 3.54 ± 0.32 L, $p < 0.005$). Short-term euglycaemia, hyperglycaemia or hyperinsulinaemia do not influence GH decay. The time-mode of GH exposure critically extends GH half-life (60min exposure $t_{1/2}$ 25.7 ± 2.1 mins; steady state (12h exposure) $t_{1/2}$ 28.5 ± 1.8 mins) describing an asymptote.

The dynamics of GH secretion are also altered: Deconvolution analysis of adolescent plasma GH profiles confirm increased GH secretion rate (median (range) $1.88(0.56-3.81)$ vs. control $0.62(0.32-1.92)$ mU/min, $p < 0.05$) and altered dominant pulse periodicity (90min versus control 135min, $p < 0.025$). Exaggerated GH secretion persists despite overnight euglycaemic clamp. Pirenzepine (anticholinergic) reduces mean GH secretion (reduction of 63%, range 9.3-82.8%) suggesting the presence of an increased cholinergic tone exists in diabetic subjects; pirenzepine did not alter periodicity which remained shortened at 90minutes. During insulin-varying clamp and somatostatin suppression, trough plasma GH levels correlate with mean plasma insulin ($r=0.52$, $p=0.016$); ambient glucose levels have no effect.

The pulse signal was differentiated: GH pulsatility (3 pulses, mean peak 31.5 (range $26.5-36.9$) mU/L) is primarily responsible for an oscillatory increase in insulin requirements occurring 135min after a GH pulse ($r=0.21$, $p < 0.001$) and lasting 240min; both pulses and continuous GH (8-10mU/L) contribute to stimulation of ketogenesis (within 1h of GH exposure); the GH pulse,

predominantly by continuous GH tone, raises free fatty acid levels. GH stimulated metabolites rise despite optimum insulin provision for euglycaemia.

GH disturbances of amplitude and pulsatility fuel insulin resistance, raising insulin requirements; hyperinsulinaemia antagonizes GH suppression summative to an existing raised cholinergic tone. Both mechanisms exacerbate GH release, aggravating GH levels further; this stimulates release of ketones, fatty acids and worsening insulin resistance leading to a spiralling cycle of deteriorating metabolic control.

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November 2002

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Author's note and Acknowledgements

Although observations on the disease 'diabetes' has been made by physicians since antiquity -from ancient writings of Arabic medicine's Avicenna (960-1037 AD) and Indian medicine's Sustruta (5th century AD) - Thomas Willis described it in 1674 (Papaspnyros 1964). Once the pancreas gland was identified (Mering & Minkowski 1890), the greatest discovery was the purification of insulin in 1922 by Banting & Best. Since then there have been many theories and discoveries. Yet still, there are many more to be made until we can ensure that our patients do not suffer the metabolic and long-term sequelae of this condition. This Thesis aims to clarify growth hormone dynamic alterations in Type I (Insulin-dependent) diabetes; and convolutes such a growth hormone profile in order to examine the relationship between this hormone and its metabolic signals. The integral message for cell starvation is unravelled. Elucidation of the metabolic alterations opens the avenue to future strategies improving short term as well as long term challenges.

The evolution of scientific knowledge is always intertwined with important past observations. I am in deep appreciation of all the careful methods and observations made by many researchers in this field.

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Author's declaration

I state that I have read and compiled the foregoing thesis. Previous peer-review journal publications relating to part of the data has been reported by BR Pal with others and provided in the bibliography. The overnight GH profiles on adolescents have been reported by J Edge and the raw data reanalysed in Chapter 5.

BRP was supported by a grant from Kabi Pharmacia, Stockholm, Sweden for the duration of the clinical studies. Serono provided the Stilamin and Kabi Pharmacia provided the Genotropin used in these studies.

Signed

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Author's Contributions, Collaborators and Supervisors

I thank the following persons who have made specific contributions toward this Thesis.

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Manuscript preparation solely by BRP

- Prepared all drafts of text
- Compiled final text, figures, tables and graphs
- Reviewed the relevant scientific literature and critical analysis of specific papers contained within this Thesis.

Clinical studies

- Recruitment of patients: BRP for GH clearance (chapter 3) and convolution (chapter 6), JAE for plasma GH (chapter 4)
- Ethics and consent: BRP
- Protocols were devised by BRP with supervision from DBD
- Studies performed: GH clearance BRP and PAE; Plasma GH profile data JAE; GH signal BRP
- Laboratory analysis of hormones and metabolites: BRP and PP

Data analysis

- All raw biochemical data formatted in disk form for analysis by BRP
- All comparison of data, subject groups using standard statistics and relevant computer programmes and final analysis by BRP
- GH decay analysis by BRP and PAE
- Fourier analysis, deconvolution analysis programmes were run by DRM
- Distribution/Probit analysis of GH profiles by BRP

Final Thesis drafts were reviewed by DBD

ABBREVIATIONS (ALPHABETICAL)

ACAC	acetoacetate
β OHB	3 β -hydroxybutyrate
Clamp	maintaining set levels of glucose or insulin in order to carry out studies
NG	Normoglycaemic clamp: blood glucose set at 5 mmol/L
NI	Normoinsulinaemic clamp: plasma insulin at 8-15 mU/L
HG	Hyperglycaemic clamp: blood glucose set at 12mmol/L
HI	Hyperinsulinaemic clamp: plasma insulin at 50 mU/L
DV	Distribution volume: the physiological space where a hormone or drug distributes in order to clear from the circulation
GH	Growth hormone implies endogenously secreted hormone or measured plasma level r-hGH recombinant human growth hormone exogenously administered
GHBP	Growth hormone binding protein
GHRH	GH releasing hormone
IGF-1	Insulin-like growth factor -1
IGF-BP	IGF binding proteins
NEFA	non-esterified fatty acids or free fatty acids
PIR	Pirenzepine: an anticholinergic drug
SRIH	somatotrophin releasing inhibitory factor or exogenous somatostatin (1-14)
SI Units	mU milliunits μ g micrograms kg kilograms GH in this Thesis is measured as mU/L. Other authors using micromols/L can use conversion factor (1 μ mol = 2mU)
$t_{1/2}$	Half-life: time taken to decay to half-plasma levels after initial entrance into blood circulation
diabetic	In this Thesis this refers to Type 1 (insulin-dependent) diabetes

CHAPTER 1

INTRODUCTION

1.0.0

GROWTH HORMONE AND PHYSIOLOGICAL ADAPTATIONS

Complex interactions exist between growth hormone (GH) and diabetic metabolism (Sonksen 1993). In adolescent subjects the precise pathology may be obscured by multiple hormone and metabolic change, manifesting with unpredictable effects in this age group. The scientific knowledge of the GH axis over the last 20 years has emerged with contradictory theories, particularly as there are multiple levels of hormone action, with complex feedback and control mechanisms, from gene expression to regulation of mature peptide action. Clarifying the subsequent role of GH secretion and action in Type 1 diabetic adolescents remains an outstanding challenge and of interest to both researchers and clinicians alike.

1.0.1 Control of normal GH secretion

GH is secreted from somatotrophs, which are found in increased numbers in the lateral wings of the pituitary, identified by specific immunological staining of large secretory granules (Wilson 1988). Now that direct measurements (animals) or specific antibody techniques are being employed (humans), a better picture is emerging of GH control. Major transmitters being involved are GH releasing hormone (GHRH), synthesised mostly in arcuate nucleus (ARC) neurones (Tannenbaum 2001), and somatostatin (SRIH), synthesised in hypothalamus within the periventricular (PVe) and ARC neurones (Carpentier 1996).

GHRH and SRIH are present in portal vessels at concentrations that stimulate or inhibit GH release and GH gene transcription (Bluet-Pajot 1998). GHRH stimulates both GH synthesis, by increasing the transcription rate of the GH gene and GH release (Mayo 2000, Cunha 2000). After binding to somatotroph-specific GHRH receptors, functionally distinct receptor proteins are generated by alternative RNA processing mechanisms (Miller 1999). In contrast

somatostatin exerts its physiological actions by its own specific receptors (six G-protein-coupled receptors) which are widely expressed in the pituitary, brain, and the periphery (Csaba 2001, Dournaud 2001).

Other neuropeptides synthesised in ARC neurones, such as galanin, or in ARC interneurons, such as neuropeptide Y (NPY), are able to modulate synthesis and release of GHRH and SRIH into the hypothalamohypophyseal portal system. Gonadal, and adrenal steroid hormones also affect the GHRH-SRIH balance and a differential distribution of sex steroid receptors in the ARC and the PVe is likely to account for the different pattern of GH secretion seen in male and female animals and humans (Van den Berg 1996). Sex-steroid hormones (oestradiol and androgens) during normal puberty augment GH secretory burst mass 1.8-3.5 fold, whereas ageing and hypogonadism mute the amplitude of pulsatile GH output.

In addition, at other hypothalamic and suprahypothalamic levels, neurotransmitters, neuropeptides such as opiates, gut hormones, corticotropin releasing hormone (CRH) and thyrotropin releasing hormone (TRH) further regulate the secretion of GHRH and somatostatin.

An ultradian rhythm in GH secretion is recognised (Wagner 1998). The secretion of GH is strongly influenced by the sleep or wake state (Brandenberger 2000). Data suggest a differential somatostatinergic control of GH release with a lower somatostatinergic tone in the hypothalamic area(s) involved in sleep regulation and sleep-related GH release than in the area controlling daytime GH secretion (Van Cauter 1998). However, the concept of a dual control of daytime and sleep-related GH secretion remains to be directly demonstrated.

Cholinergic muscarinic pathways play an important role in GH secretion (Goni 1997) particularly in the sleep release of GH (Peters 1986). Previously muscarinic agonists were shown to stimulate basal GH release and enhance GH responses to GHRH (Massara 1986, Torsello 1988, Panzeri 1990). Conversely, antagonism of endogenous cholinergic pathways, with muscarinic receptor

blocking drugs such as mescolapine or atropine or pirenzepine causes a striking reduction in the slow wave sleep related GH release (Peters 1986) and virtually abolishes the GH responses to L-dopa, apomorphine, glucagon, FK-33-284 arginine, physical exercise, clonidine and GHRH (Massara 1986). However, other authors have found that pirenzepine completely abolished the normal sleep-induced GH surge *without* affecting plasma GHRH levels (Page 1987a). Furthermore, the GH response to insulin-induced hypoglycaemia is largely spared during cholinergic muscarinic receptor blockade, although the reasons for this are unclear (Evans 1985a). Thus, the cholinergic influence on GH control is complex.

Some studies have shown that muscarinic antagonists block an inhibitory effect exerted by acetylcholine on SRIH release from the hypothalamus (Richardson 1980, Mazza 1994). Others have shown that pirenzepine does not exclusively act via release of somatostatin, but may act through inhibition of GHRH function. Such increase in SRIH concentration could reduce or block the stimulatory effect of exogenously administered or endogenously released GHRH on GH secretion. In contrast, GH responses to hypoglycaemia may be mediated by reduced SRIH release that might override the effects of cholinergic muscarinic blockade. In partial support of this hypothesis the inhibitory effect of atropine on GH responses to GHRH in normal rats was abolished by pre-treatment with anti-somatostatin antibodies (Locatelli 1986). Although muscarinic and nicotinic cholinergic pathways generally play opposite regulatory roles (facilitatory and inhibitory respectively), the effect on GH secretion may be different *in vivo* compared with *in vitro* models.

1.0.2 The effects of metabolic factors on GH secretion

Modulation of GH secretion by humeral factors such as glucose, free fatty acids and ketone bodies were previously thought to leave the basic temporal pattern of GH secretion relatively unaffected (Quabbe 1985).

Glucose deficiency causes a rise in plasma GH concentration which has an important role in glucose counter-regulation (Wurzbürger 1990). The mechanism by which hypoglycaemia causes GH release is unclear: some authors suggest the pathway is probably largely independent of GHRH; others have shown that GHRH and hypoglycaemia do have additive effects on GH release when administered together (Page 1987b). After prior GHRH administration, the subsequent GH responses to GHRH are abolished while GH responses to hypoglycaemia are still preserved and seemed to be enhanced (Vance 1986). Vance et al (1986) provided some evidence that hypoglycaemia increases GH by reducing somatostatin release from the hypothalamus. However, the mechanism that predominates is still not clear as it is possible that there is contribution both from release of hypothalamic GHRH or other factors with GH releasing activity, and by reduction of SRIF release.

Whereas hypoglycaemia stimulates GH release, an **increase in blood glucose** levels in normal subjects reduces basal and stimulated GH levels. Elevation of the blood glucose concentration reduces the GH response to a number of stimuli (Penalva 1989) and this suggests that hyperglycaemia may act by eliciting somatostatin release from the hypothalamus. Non-suppression by hyperglycaemia has been demonstrated in the GH response to arginine, exercise, sleep, L-dopa and GHRH-mediated secretagogues.

In vivo studies in normal subjects suggests that **insulin** does not have a major direct role in GH regulation. Early studies using step wise increases in insulin infusion in normal adults, but without taking precautions to maintain a stable blood glucose, failed to demonstrate a change in GH concentration until the blood glucose had fallen by fifty per cent (Sonksen 1972). However, although there was no immediate GH response to insulin when the hypoglycaemia was

blocked by glucose administration, a late rise in plasma GH was seen some three hours after a bolus of insulin with blood glucose maintained at 4 mmol per litre (West 1977). This rise is analogous to the late rise seen after oral glucose ingestion and suggests that this response may be mediated either by a direct stimulatory effect of insulin or by an altered glucose flux in tissues after the initial accelerated glucose disposal. Which of these events have taken place has not been established.

Free fatty acid administration to normal subjects reduces GH release: this effect is probably due to increased somatostatin release since free fatty acids reduced GH responses to GHRH in normal rats but not in rats treated with anti-somatostatin antibodies (Imaki 1986). However, this does not exclude an additional direct pituitary action (Casanueva 1987).

Administration of free fatty acids (NEFA) to normal subjects reduces the GH response to a variety of different stimuli including hypoglycaemia, physical exercise, L-dopa, clonidine, arginine, sleep and GHRH which is also seen in patients with elevated NEFA and/or triglycerides (Imaki 1985). Lack of free fatty acids is also a stimulus to GH release (Quabbe 1972).

Ketone bodies also seem to be involved in the modulation of GH release, since ketone body administration will almost entirely abolish the GH response to lack of free fatty acids (Quabbe 1983).

Insulin-like growth factor-1 (IGF-1) acts as a negative feedback in the somatotrophic axis inhibiting GHRH and stimulating somatostatin at the hypothalamus or pituitary or both.

Daily GH hormonal profiles are thus the product of a complex interaction between the output of the circadian pacemaker, periodic changes in behaviour, light exposure, neuroendocrine feedback mechanisms, gender, age and the timing of sleep and wakefulness. The interaction of these factors can affect hormonal secretory pulse frequency and amplitude. The variable interactions between GH and other hormone and metabolic factors all require consideration particularly when examining subjects in-vivo.

1.0.3 GH pulsatility

In the normal state, the pulsatile nature of GH secretion from the pituitary gland causes circulating GH levels to fluctuate widely. During much of the time, plasma GH is undetectable (trough or baseline levels) by current radioimmunoassays and punctuated by secretory episodes, resulting in plasma peaks of varying height, which primarily occur at night. Episodic pituitary hormone secretion generally reflects episodic stimulation by the hypothalamic releasing factors. However, direct measurements are difficult and in humans essentially impossible, as dilution of the small portal circulation into systemic blood reduces peripheral concentrations of hypothalamic releasing factors to unmeasurable values. Thus, the assessment of the pattern of GH pulses in peripheral blood is usually the only clue to the pattern of hypothalamic secretion although this is clouded by the distortions of clearance, mixing, responsiveness of the pituitary and measurement error. Methods of examining hormone secretory profiles are many (Bright 1999, Langendonk 2001) and the various methods are appraised in Chapter 4.

In human subjects GH secretion is known to be oscillatory (Ovesen 1998) but the proposed mechanism of pulsatility differs between authors. Originally studies in perfused rat pituitary cells have led to the postulation that hypothalamic somatostatin withdrawal sets the timing and duration of the GH pulse and GHRH activity sets the amplitude of the pulse (Tannenbaum & Ling 1984, Weiss 1987). When continuous or intermittent GHRH infusion was given in adult male human volunteers, augmentation of consistent repeatable GH responses only occurred after withdrawal of somatostatin, suggesting that the generation of regular pulsation of GH is dependent on somatostatin changes (Hindmarsh 1991). Similarly, acromegaly is caused by excess GH release but remains episodic in the presence of continuous GHRH suggesting that hypothalamic somatostatin (SRIF) secretion is preserved (Gelato 1990).

Authors previously concluded that a concomitant rise in GHRH levels is required during periodically quiescent somatostatin levels (Plotsky & Vale

1985, Cronin 1987, Ho 1993). In contrast, when using a continuous high dose synthetic SRIH (Octreotide) to create a constantly raised somatostatinergic tone and a sensitive chemiluminometric assay to measure endogenous GH, both GH pulse frequency and the response to GHRH and GHRP-6 stimulation was unchanged compared to a constant saline infusion and these authors concluded that somatostatin withdrawal was therefore not critical to GH pulse initiation (Dimaraki 2001). Conversely, others have shown that GH pulsatility can be generated in normal volunteers by the combination of continuously raised GHRH and intermittent SRIH (Achermann 1999). This suggests that GHRH stimulates GH synthesis and replenishment of stored GH pools, even during high SRIH tone when GH is effectively modulated by somatostatinergic restraint. Paradoxically, intermittent somatostatin also facilitates somatotrope cell responses to secretagogic stimuli, thus amplifying pulsatile GH secretion. The neurophysiological regulation of GH is thus complex (Veldhuis 2001).

Of the total GH secreted, 8-12% manifests as a low basal rate of GH release, controlled by facilitative action of GHRH and GHRP and inhibitory action of SRIH. The abrupt rise of the GH pulse itself maintains its own homeostasis by a time-delayed signal feedback to the central regulatory centres, subsequently on ARC neurones acting via specific GH receptors, to activate SRIH and to subdue GHRH (Peng 2001). Thus GH itself is able to inhibit the amplitude of GH secretory episodes and to increase their frequency (Nass 2000). This autoregulatory loop contributes to the time-dependent physiological pulsatile dynamics of the GH axis. More slowly, the IGF-1 feedback axis at varying IGF-1 concentrations dampens GH secretory pulse amplitude by a delayed negative-feedback action. The mechanism of action of SRIH, as an inhibitor is therefore complex as it may directly inhibit GH release from the pituitary, or act at the level of insulin-like growth factor-I, IGF-I or even at the level of GH releasing hormone. Thus, GHRH and GHRP are stimulatory and SRIH, GH and IGF-1 are inhibitory feedback signals to GH pulsatility.

1.0.4 GH actions

Endogenous GH consists of 191 amino acids and both growth-promoting and diabetogenic properties are intrinsic to the molecule.

The **amplitude and frequency** of the GH pulse signal has been shown to be advantageous for growth (Bick 1992, Achermann 1999). Data from rats also suggest that **trough** concentrations are as important as peak concentrations when interpreting the GH signal (Agrawal 2001), particularly as trough levels may relay alterations of the **GH binding protein (GHBP)**. The GHBP is thought to correspond to the extracellular, ligand-binding domain of the GH receptors (GHR) in tissues, reflecting GH receptor status (Amit 2000). The high affinity GHBP is the circulating ectodomain of the GHR and is produced in many tissues, particularly liver, either by alternative GHR mRNA splicing (rodents) or by proteolytic cleavage from the GHR (humans) and parallel GHR expression (Baumann 2001). In general low levels of GHBP occurs in conditions associated with GH resistance such as malnutrition and diabetes (Menon 1992, Radetti 1997), conversely obesity is associated with elevated levels. GHBP binds about half of the circulating GH under basal conditions but is easily saturated at high GH levels. It has complex functions including a circulating buffer/reservoir function for GH, prolongation of plasma GH half-life, competition with GHRs for GH, and probably unproductive heterodimer formation with the GHR. The net effect of these partly enhancing and partly inhibitory functions on GH action *in vivo* is complex, difficult to ascertain and varies with age and between species.

In the male rat GH secretion is characterised by regular GH peaks of high amplitude, and their GH receptors and GH-binding protein are low whereas female rats have a more continuous secretion and their GH receptors and GH-BP are high. The mechanism appears to be that each surge of GH results in individual pulse-related turnover of receptor internalisation and recycling and this is accompanied by a parallel increase of serum GH-BP

activity, which is competitive and cyclical. The GH and receptor wave is then responsible for an individual secretion pulse of IGF-1 (Bick 1990). Furthermore, this author showed that when physiological doses of GH were infused into hypophysectomised rats either as pulses (1.5u/kg/day, every 5minutes for 3 hours for 5 days) or continuously, the pulsatile GH pattern maximally stimulated growth and induced a cyclical change in the levels of hepatic cell membrane receptors and circulating GH-BP, whereas GH receptors and GH-BP were enhanced maximally by continuous GH levels and weight gain was poor (Bick 1992).

The mechanism of the **nitrogen retaining** (anabolic) effects in humans appears to be that the GH pulse signal triggers tissue-specific gene expression, promoting skeletal and muscle growth. An abrupt rise in circulating GH concentrations stimulates rapid internalization of the GH receptor in peripheral target tissues and evokes second messenger nuclear signalling. Discrete GH peaks stimulate linear (skeletal) growth and drives muscle IGF-1 gene expression more effectively than basal GH exposure. Assessment of prepubertal children has also suggested that peak levels of GH influence growth rate and the IGF-1 axis, although elevated trough levels do influence growth, but only when GH peaks are low (Achermann 1999).

Removal of the first 42 residues of the intact hormone, the amino terminal portion of hGH, enhances its **diabetogenic properties** (Lewis, 1991). Since the late 1920's it has been recognised that pituitary extracts and later purified GH are capable of producing major alterations in the metabolism of fat and carbohydrate. Although these early studies show that GH is a powerful diabetogenic hormone, the picture became rather less clear with the demonstration of both insulin-like and insulin antagonistic effects of GH (Davidson 1987). MacGorman and her colleagues, using GH infusion and providing constant background insulin levels in humans, showed that GH has an **early transient insulin-like effect** (MacGorman, 1981) which was evident on both the liver, reducing endogenous glucose production, and peripheral tissues

increasing glucose uptake but this effect was reversed in two hours. Following a subsequent glucose infusion, increased hyperglycaemia compared with control was observed due to impaired suppression of hepatic glucose production and to reduced glucose clearance. In another study using normal subjects, longer term GH administration for four days produced significant carbohydrate intolerance but with no change in insulin binding to monocytes, suggesting that GH causes insulin resistance.

GH also has a **lipolytic effect** (Richelsen 1997). Free fatty acids (NEFA) concentrations increased two hours after GH suggesting that the lipolytic activities of GH are intrinsic properties of the molecule administration (Sirek 1967, Hart 1984). However, other studies show that the lipolytic activity may only become apparent after either modification of the molecule in vivo or activation of a lipolytic intermediate and dispute the notion that GH itself causes lipolysis. For example, in animals, amplification of adipose tissue response by GH (bovine somatotrophin) was seen *only* after excitement (intensive handling) or epinephrine administration, suggesting that adrenergic stimulation of adipose tissue promoted NEFA response (Boisclair 1997). Thus, this amplification of adipose tissue response must be considered when conducting intensive studies as even the minimal excitement associated with blood sampling can confound results regarding lipid mobilization.

GH also has **acidogenic and basogenic actions**. Acid-base and nitrogen homeostasis are interdependent through the co-ordinated action of growth hormone/IGF-1 on substrate fluxes. Growth hormone's acidogenic action, as a consequence of promoting fatty acid utilization yields protons required for driving hepatic glutamate production. Subsequent peripheral retrieval of the salvaged glutamate requires insulin-like growth factor-1 (IGF-1) activated uptake and acid-base homeostasis. In addition to the nitrogen sparing acidogenic effect, GH is also basogenic in combination with IGF-1 and acts on the kidney to maintain acid-base balance (Welbourne 1997).

1.0.5 GH secretion during fasting

The well-established physiological metabolic responses to GH can change markedly in conditions of reduced nutritional supply or metabolic stress.

Work in animals indicates alterations of the **GH secretory pattern** by fasting and prevailing metabolic conditions (Tannenbaum 1979, Breier 1986). Withdrawal of food in both male and female guinea pigs changed the GH secretory pattern dramatically (Fairhall 1990). The normal episodic GH secretory pattern (large GH peaks occurring at 3.6 ± 0.4 h intervals over a low (0.5-1.5 $\mu\text{g/L}$, 1-3 mU/L) baseline secretion was altered to a pattern of more continuous GH output, characterised by a 10-fold elevated baseline secretion (5-15 $\mu\text{g/L}$, 10-30 mU/L) with no large secretory episodes or troughs. However, the elevated GH secretion in these fasted animals could be inhibited by continuous infusion of somatostatin.

Augmentation of GH release pattern is also seen in humans. During their studies in humans, Ho KY (1988) showed that while the increase in integrated GH concentrations had occurred by the first fasting day there was a further increase in the non-pulsatile component of GH release from four fold on the first fasted day to ten fold on the fifth day.

Fasting (or calorie deprivation) also augments GH response to GHRH: In animal studies, somatostatin, given at low dose, abolishes the GH response to GHRH in the fed state but not in the fasted state. Higher doses of SRH were needed to abolish the GHRH response in the fasted state. However, pirenzepine (anticholinergic) administration lowered the GHRH response in both fed and fasted states, although this inhibition was less evident in the fasted state (Rigamonti 1998).

Thus, it has been suggested that during fasting there appears to be an augmented GHRH secretion and somatotrope refractoriness to somatostatin to enable this enhanced secretion. Metabolic abnormalities associated with food deprivation may also act directly on the hypothalamus on the neuroendocrine control of the releasing factors that regulate GH (Laughlin 1996, Stoving 1999). For

instance, insulin injections (1, 2 or 6 u iv) inhibited spontaneous GH pulses in normally fed animals, but has little effect on the high continuous GH tone during fasting. This and other metabolic factors need consideration when examining neuroendocrine control.

1.0.6 GH action during fasting

The presence of a nutrition dependent component of GH action would be beneficial to man since anabolic processes can proceed only in the presence of adequate nutrition. Decreased nutrition not only leads to elevated GH secretion, but it also reduces hepatic GH receptor (GHR) number and plasma levels of IGF-1. Circulating glucose and IGF-1 are both known inhibitors of GH secretion and anabolic effects of IGF-1 would have to be minimal during starvation since circulating and tissue concentrations of this growth factor are low. IGF-1 levels are restored with refeeding but both adequate protein and energy intake are necessary to return IGF-1 levels to normal (Caregaro 2001). Elevated GH normally improves the partitioning of nutrients by increasing protein synthesis and decreasing protein degradation. Changes in the relative concentrations of IGF binding proteins (IGFBPs) in plasma also have a role in muscle protein synthesis (Svanberg 2000).

The qualitative changes in the pattern of GH release seen with prolongation of fasting may also play an important role in mediating the well-documented switch from a predominantly gluconeogenic to a lipolytic source of metabolic fuel. The first relates to the maintenance of glucose supply through an increase in hepatic glucose production and second the provision of an alternative energy source by increasing fat mobilisation and oxidation thereby indirectly sparing body protein. Authors disagree as to whether GH directly or indirectly mediates these homeostatic mechanisms.

The role of **fat-derived substrates** in the regulation of gluconeogenesis during fasting has been examined in normal 4 day fasted volunteers treated with Acipimox (antilipolytic drug) or placebo for 8 hours (Fery 1996).

During both fasted and non-fasted states, **free fatty acids** did not exert a direct stimulatory role on gluconeogenesis, but acted indirectly through an ability to modulate hormone changes, particularly growth hormone and insulin. **Ketone-clamp** experiments in Acipimox-treated fasted subjects also showed that during fasting, both ketones and NEFA suppressed gluconeogenesis but by different mechanisms (Fery 1996). The mechanism is thought to be that during starvation ketoacid production, as the consequence of incomplete fatty acid oxidation and ketone excretion, swamps the basogenic limb of GH and full-blown metabolic acidosis prevails. Under this condition growth hormone's effectiveness in sparing nitrogen for anabolic processes is curtailed as glutamate (emanating from the liver) and glutamine (derived from muscle proteolysis) are directed to the kidneys, supporting **ammoniogenesis**: nitrogen balance is now sacrificed for acid-base homeostasis. Underlying this state is an intracellular acidosis that may contribute to insulin resistance and developing hyperglycaemia in response to growth hormone.

This complex interplay of fasting and nutrients triggers a dramatic alteration in GH secretion and GH action.

1.0.7

GROWTH HORMONE AND TYPE 1 DIABETES

1.0.8 GH secretion

GH levels may be high in subjects with Type 1 diabetes (Hansen 1970, Salgado 1996). Comparison of GH pulsatility in twelve hour plasma GH profiles in subjects with Type 1 diabetes with those of non-diabetic controls have yielded conflicting results: some authors suggest an exaggeration of GH release with an increase in the number of GH pulses in diabetic subjects (Asplin 1989, Halldin 1998, Asplin, 1989) while others saw no difference (Molnar 1972). The **amplitude** of the GH pulses was consistently increased in most studies (Horner 1981, Batch 1992, Edge 1990), as was the mean integrated twenty-four hour GH concentration (Hayford 1980, Zadik 1980). Edge (1990) described overnight GH profiles in adolescent subjects with Type 1 diabetes and compared findings to puberty-matched control subjects. This author confirmed that the higher circulating level of GH in Type 1 diabetes is due to both an increased GH pulse amplitude (mean peak (SD) 35.5 ± 4.2 mU/L) and a higher baseline (non-pulsatile component 5.01 ± 0.62 mU/L). Other authors showed that while a difference in raised GH level was seen in prepubertal diabetic subjects, when compared with controls, there was no difference during puberty (Nieves-Rivera 1993). The current mechanism of this hypersecretion is accepted as mediated by feedback drive from low insulin-like growth factor (IGF-1) (Clayton 1994, Savage & Dunger 1996, Bereket 1999). The **first (initial) phase** of insulin-dependent diabetes mellitus is characterised by the absence of insulin, high GH levels and low plasma IGF-1. The pituitary GH response to exercise and other stimuli is exaggerated. The **second phase** of disease ('C-peptide positive phase') is characterised by the return of some residual beta-cell insulin secretion, increased levels of GH compared to non-diabetic subjects, physiological IGF-1 levels and near normal pituitary GH responses to different agents. The **third**

phase of the disease is characterised by complete loss of endogenous insulin secretion, very high plasma GH levels, low normal plasma IGF-1 but impaired hepatic IGF-1 generating capacity. The control mechanisms of pituitary GH secretion (long loop negative feedback and auto-feedback) are disturbed (Wurzbürger 1996). Despite this accepted mechanism, the neuroendocrine mechanisms regulating GH secretion in Type 1 diabetes are still debated (Miller 1992, Müller 1995, Salgado 1996).

1.0.9 Mechanism of GH hypersecretion

Insight into the hypothalamic control of GH in human subjects with diabetes has previously been gained indirectly and remains contradictory. The dilemma is partly because previous studies have used animal models, chiefly the rat, and induced experimental diabetes. Animal work has established that SRIF, rather than GHRH, plays an active role in modulating GH secretion in diabetic rats (Ndon 1992). In a rat model of diabetes (Goto-Kakizaki or GK rat), Ismail (1995) has shown that somatostatin release mediates a blunted GH response to GHRH in GK rats and that reduced hypothalamic cholinergic signalling to the somatostatinergic neurone may mediate the increase in somatostatin release. This view is supported in part by the results from *in vitro* studies in which cholinergic muscarinic blockade with pirenzepine (PIR) caused dose-related stimulation of somatostatin release from normal rat hypothalami but was without effect on GK rat hypothalami. The cause of this alteration in hypothalamic function is, at present, unknown.

Extrapolation of these findings to the human condition is not always valid and often adds confusion due to the intrinsic differences between the species, particularly in relation to the physiology of GH. For example, GH induces its own receptors in the rat (Baxter 1984a) but down-regulates its receptors in man (Murphy 1984). Stress and hypoglycaemia are both accompanied by suppression of GH levels in the rat (Painson 1985) again in contrast to the observed effects in man. Experimental diabetes in the rat is characterized by

low levels of GH and Insulin-like growth factor-1 (IGF-1) (Maes 1983) but in man, poorly controlled diabetes is characterized by normal or low IGF-1 levels despite raised circulating levels of GH (Amiel 1984). A better understanding of the precise role of GH in controlling diabetic metabolism can be obtained only by observations in human subjects *in vivo*.

GH secretion in humans has been previously studied after administration of various **pharmacological stimuli**, either singly or in combination. Observations have shown that GH responses in diabetic adult subjects are increased following the administration of a number of pharmacological and physiological stimuli including hypothalamic stimuli (Topper 1985) and pituitary stimuli (Giampietro 1987). However, it is clear from clinical studies that responses to exaggerated pharmacological stimulation may not represent subjects in their usual physiological state.

The **amplitude of GH pulses** in Type 1 diabetic subjects was increased in most studies and it has been noted that the baseline GH concentration from which the GH pulses arise, was higher in diabetic compared to non-diabetic subjects. The mechanism behind this remains contradictory. Some authors suggest that exaggerated GH levels are due to primarily to GHRH-mediated effects. In contradiction, others have shown that GH responses to GHRH did not change either during fasting or in subjects with Type 1 diabetes. Furthermore, data suggest that independently, both GHRH and GH-releasing peptide-6 (GHRP-6) releasing mechanisms are unaltered in subjects with Type 1 diabetes (Villas-Boas Weffort 1997). The enhanced spontaneous GH secretion is therefore unlikely to be due to marked alterations in somatotrope responsiveness to hypothalamic GHRH (Kopelman 1988).

Although some studies in subjects with Type 1 diabetes related the problem of increased GH release simply to poor **glycaemic control** (Press 1984a), others showed no short term effects of ambient glucose concentration on GH responsiveness (Ismail 1991). Acute changes in metabolites may also produce different effects on GH secretion when compared with chronic

changes and this needs to be explored further in subjects with Type 1 diabetes where alterations in glucose, insulin and intermediary metabolism are in constant flux. As the various mechanisms of GH control are deduced from animal models and elucidated in clinical studies primarily involving adults, it must be remembered that children and adolescents with Type 1 diabetes may have alterations in the control of GH release and require careful assessment. A further study recently has showed that fasting insulin levels and abdominal visceral fat were consistently important predictors of integrated 24-hour GH concentrations, independent of age group, gender or other variables (Clasey 2001). Thus a powerful relationship, with bi-directional feedback, exists between fasting insulin, visceral fat and IGF-1.

Thus, equivocal evidence exists regarding the sensitivity of the pituitary gland in Type 1 diabetes: GHRH may be increased or reduced suppression of GH occurs due to metabolic or hormonal factors causing a reduced somatostatin release.

1.1.0 Impact of GH hypersecretion

Although some authors claim that the actions of GH is not of critical physiological significance (Laurson 1995), there is ample evidence to suggest that discrete pulse alteration influences somatic and metabolic changes. Some previous studies in Type 1 diabetic subjects concluded that glucose disposal did not correlate with counterregulatory hormones or plasma NEFA, or antecedent glycaemic control (Davis 1992) but others showed that raised GH is accompanied by a decrease in insulin sensitivity in the liver and muscle (Moller 1991). Consistent evidence now shows that increased plasma concentrations of GH in Type 1 diabetes impairs metabolic control (Press 1984a, Orskov 1985, Gerich 1986), is implicated in the development of the dawn phenomenon (Campbell 1985a) and be relevant to the development of microvascular disease (Olsen 1999, Flyvbjerg 1997, Cummings 1998, Landau 1998) that often becomes evident at around the time of puberty.

The mechanism of in-vivo GH effects on carbohydrate and lipid metabolism is still unclear in Type 1 diabetic subjects. There is good evidence that circulating GH has effects on **glucose metabolism**. Hypoglycaemia is observed in GH deficient children who also demonstrate low insulin levels to a standard glucose challenge while maintaining normal blood glucose levels (Hopwood 1975). GH replacement in these patients normalises plasma glucose levels and abolishes hypoglycaemia by increasing hepatic glucose production (Bougnieres 1985).

The **insulin-like effects** of GH involve enhanced glucose utilisation (probably at the glucose transport step) and anti-lipolysis. They can be demonstrated in-vitro but are clearly easier to show if the tissue has had little or no recent prior exposure to GH. As the tissues in diabetic patients are exposed to frequent bursts of endogenous secretion of the hormone, the insulin-like effects of GH are not important physiologically. The **anti-insulin-like effects** of GH are more important and involve impaired glucose utilisation, inducing a refractory state to the insulin-like effects of the hormone and stimulate lipolysis.

Insensitivity to the actions of insulin is now believed to be a common feature of Type 1 diabetes (Olefsky 1985). A reduction of 30 to 60% in the metabolic response to a given insulin stimulus has been reported in diabetic adults when compared with matched normal subjects (Simonson 1985). The development of insulin resistance may be dependent on duration of the diabetes as it is present before treatment is started (Yki-Jarvinen 1986), disappears after treatment of less than three months duration (Gray 1986) and is significantly greater after five years than one year duration.

The mechanism of the **insulin resistance** in Type 1 diabetes is still unclear (Schliess 2000), although GH and other counter-regulatory hormones such as epinephrine (Walters 1992) and androgens (Ebeling 1995) have been implicated. Further evidence regarding the role of metabolites is also emerging: Insulin resistance with inhibition of glucose disposal in Type 1 diabetes is also observed after hyperglycaemia (Del Prato, 1997) and hyperlipidaemia (Balkau 1998).

GH stimulates **lipolysis**, providing glycerol as a substrate for gluconeogenesis and free fatty acids (NEFA) which impair tissue glucose oxidation (Johnston 1985, Neilsen 2001). In normal men high fatty acids have been shown to affect the ability of insulin to suppress endogenous glucose production (Bisschop 2001). Further studies in Type 1 (insulin-dependent) diabetic subjects suggest that the mass-action effect of free fatty acids inhibits insulin mediated glucose disposal in muscle and stimulates gluconeogenesis in the liver, leading to insulin resistance. Current thinking shows that even physiological increases in plasma NEFA levels cause insulin resistance in both diabetic and non-diabetic subjects (Boden 2001).

While increased nocturnal lipolysis has been associated with elevated GH in the absence of insulinopenia or hyperglycaemia (Hagstrom-Toft 1997), some authors have shown that peripheral insulin resistance was only observed in the presence of a *combined increase* in total lipid and NEFA oxidation but not during an isolated increase in NEFA oxidation, whereas hepatic insulin resistance could be induced even by a moderate increase in NEFA availability (Laville 1995). NEFA causing hepatic insulin resistance results in increased rates of endogenous glucose production in relationship to the prevailing degree of insulinaemia. However, this is not the only mechanism. GH may induce insulin resistance through non-NEFA dependent mechanisms but these cellular mechanisms remain to be elucidated.

A complex picture thus exists regarding the role of GH, glucose, insulin and NEFA in the metabolic derangement of insulin resistance.

1.1.1 Insulin resistance, GH and Puberty

During puberty there is an amplified secretory burst mass resulting in heightened GH pulse amplitude and duration (Zadik 1985, Veldhuis 2000). Sex steroids have also been associated with the different facets of altered GH pattern – an elevated basal GH secretion and pulsatility is associated with oestrogens (Pincus 1996) and amplified GH secretory burst mass and higher plasma IGF-1

associated with rise in testosterone (Veldhuis 1996a,b,1998). However, short term fasting (Maccario 2000) abolishes these gender differences. Distinctive **mechanisms of GH release** may be altered during puberty but the evidence from normal subjects is contradictory. For example, repeat GHRH administration in a group of children in puberty did not lead to inhibition of the subsequent GH response, and may even have increased it (Ghigo 1989); whereas in a study of normal adolescents, GH responses to GHRH did not change throughout puberty and was similar to those of adult women except for in boys where the response in mid-puberty was lower than those in either pre-puberty or adult men (Gelato 1986).

There is evidence that a **physiological adaptation in insulin resistance** occurs with the onset of puberty (Moran 1999, Hoffman 2000). A combination of hormonal and metabolic factors may contribute to this adaptation (Goran 2001). Insulin, being an anabolic hormone, is essential for appropriate tissue development, growth and maintenance of whole body homeostasis. Insulin regulates glucose homeostasis at many sites, reducing hepatic glucose output (via gluconeogenesis and glycogenolysis) and increases rate of glucose uptake into striated muscle and adipose tissue. The cellular site of the insensitivity to insulin action appears to be subsequent to insulin binding with its glyco-protein receptor on the cell surface. The clearance of circulating glucose in tissues depends on insulin-stimulated translocation of glucose transporter (GLUT-4) isoform to the cell surface. Insulin resistance occurs when either the normal circulating concentrations are insufficient to regulate these processes appropriately or the maximal response to insulin is reduced by a rate-limiting step in insulin action (Scheen 1996).

Insulin doses during puberty in subjects with Type 1 diabetes, even when adjusted for body weight, often exceeds those in both younger children and adults. These changes parallel those observed in normal children where despite comparable levels of fasting blood glucose, normal adolescence have higher fasting concentrations of insulin than those found in younger children and

adults. These findings in both diabetic and non-diabetic adolescents could be explained by a reduction in insulin sensitivity during puberty.

Amiel and colleagues have provided some evidence for this by using the euglycaemic clamp technique (Amiel 1986), they showed a marked reduction (by 33 to 42%) in insulin stimulated glucose metabolism in pubertal compared with pre-pubertal diabetics, and noted similar changes in normal siblings. Diabetics at all ages were again remarkably insulin resistant compared with matched normal control. It seems likely that these changes in insulin resistance are related to hormonal changes, which are occurring during puberty. This includes elevated levels of growth factors and sex steroids and increased pulsatile GH release.

Several syndromes of hyperandrogenaemia are associated with insulin resistance (Prelevic 1997) and plasma dihydroepiandrosterone sulphate (DHEAS) concentrations have been reported to correlate with fasting and glucose stimulated insulin responses during puberty but not in pre-puberty (Smith 1989a). However, a simple relationship between the levels of sex steroids and insulin resistances is unlikely since adults have a lower insulin resistance than during puberty (Caprio 1989), despite having higher circulating levels of sex steroids. Furthermore, the rate of insulin mediated glucose metabolism in normal women does not appear to be affected by the phase of the menstrual cycle, despite large differences in sex steroids levels. It is much more likely that sex steroids may act synergistically with other factors to contribute to the insulin resistance of puberty.

Another possible mediator is IGF-1 and indeed the reduced insulin sensitivity during normal puberty is associated with raised IGF-1 concentrations (Caprio 1989). This does not however imply a causal relationship but is more likely to be the result of parallel response to another factor such as increase in GH levels. The IGF-1 molecule has structural homology with insulin and has insulin-like metabolic effects *in vivo* and so is unlikely itself to cause insulin resistance (Guler 1987). However, in diabetics

there is evidence for the presence of inhibitors of IGF-1 action, particularly IGFBP-1 (Taylor 1987). These may also inhibit some of the actions of insulin (Avasthy 1986) and be a contributor to insulin resistance although it remains to be determined whether these inhibitors are important during puberty.

Insulin requirements increase because of rapid growth in puberty, even when corrected for body weight. Amiel and her colleagues demonstrated an inverse correlation between mean 24-hour levels of GH and the insulin stimulated glucose metabolism during their clamp studies, when diabetic and non-diabetic adolescents were analysed together. They suggested that the physiological increase in insulin resistance at puberty may be related to GH levels (Amiel 1986). In the study of Beaufrere and colleagues in a group of eight well-controlled diabetic adolescents a correlation was seen between the insulin infusion rate required to maintain normoglycaemia in the early part of the night and nocturnal GH release (Beaufrere 1988). This observation was also confirmed by others when a direct relationship with mean plasma GH levels and plasma free insulin levels was seen (Edge 1990a, Hindmarsh 1988a). These studies strongly imply that GH is the mediator of the insulin resistance of puberty.

Other factors such as insulin antibodies, degree of insulization and exercise may affect insulin resistance in diabetes but there is no trend in these parameters with puberty thus they are unlikely to be the cause of the substantial increase in insulin resistance during puberty compared with younger children and adults.

The major **site of insulin resistance** is likely to be skeletal muscle. Plasma free insulin concentrations achieved with the hyper-insulinaemic clamp technique are sufficient to effectively suppress hepatic glucose production and hepatic glucose uptake only accounts for a small part of the clamp glucose disposal (DeFronzo 1983). Since adipose tissue accounts for only a small proportion of glucose metabolism and insulin sensitivity is directly related to muscle mass and inversely proportional to adiposity (Yki-Jarvinen 1983) skeletal muscle is

probably the major site of insulin sensitivity. Thus, although hepatic and peripheral insulin insensitivity has been reported in Type 1 diabetes, the majority of clinically important glucose resistance must reside in peripheral tissues. Circulating GH is therefore implicated in the insulin resistance of puberty and thus are additive with the effects of diabetes on insulin insensitivity during puberty.

1.1.2 Nocturnal GH, insulin requirement and Dawn Phenomenon

Increased plasma GH levels may have insulin antagonistic effects overnight with greatest manifestation in the early morning hours. Clarke and colleagues (1980) reported that insulin requirements for normoglycaemia in six adults with Type 1 diabetes were two to three fold higher between 06.00-09.00 hours than between 24.00-06.00 hours. This increase in basal insulin requirement was termed the '**Dawn Phenomenon**'. Schmidt and co-workers also used the term to describe the early morning rise in blood glucose concentrations on conventional insulin regimes, when increases in insulin requirements were not being met (Schmidt 1981). Following these reports the dawn phenomenon was demonstrated in many studies of adults with Type 1 diabetes (Skor 1983) and with non insulin-dependent diabetes (Bolli 1984b).

However, many of these early studies were carried out using the artificial pancreas, which was later shown to produce aggregation and loss of available insulin from the infusion solution after prolonged use (Brennan 1985). Because of this artefact it appeared that there was an increase in insulin clearance that contributed to a major extent to the increased requirement in the dawn hours (Dux 1985).

Studies using a Harvard Pump, which did not demonstrate the loss of insulin from the insulin infusion, have shown that dawn phenomenon does occur in adults with Type 1 diabetes but without the increased insulin clearance or fall in plasma insulin concentrations seen with the artificial pancreas (Campbell 1986).

The increase in insulin requirement was actually less than one third of that found in previous studies (De Feo 1986). These studies concluded that changes in insulin sensitivity are implicated in the pathogenesis of the dawn phenomenon and demonstrated that the dawn phenomenon was very reproducible from day to day in adults with Type 1 diabetes (De Feo 1986).

Several studies measuring overnight blood glucose and plasma insulin profiles in normal adults have failed to demonstrate any change in dawn parameters (Simon 1988). In contrast, an increase in **hepatic glucose output** in the early morning has suggested that a dawn phenomenon does occur in normal adults. Bolli and colleagues showed that rates of glucose production, glucose utilisation and insulin secretion all increase after 05.30 hours in normal volunteers suggesting the presence of a dawn phenomenon initiated by increase in glucose production (Bolli 1984a). Other authors report an increase in plasma insulin clearance similar in both non-diabetic and diabetic adolescent subjects, while normal blood glucose levels did not change during the dawn period (Arslanian 1992). However, the dawn rise in blood glucose levels has been reported in diabetic adolescents during a constant rate overnight infusion of insulin (Edge 1990b, Dunger 1991).

The search for possible causes of this early morning reduction in insulin sensitivity initially focused on **cortisol** since the dawn phenomenon occurred at the time as the physiological pubertal rise in cortisol concentrations (Trumper 1995). However, abolition of the cortisol rise with metyrapone and dexamethasone did not inhibit the early morning increase in insulin requirement (Skor 1983), and the rise of cortisol appears to be too late to affect early morning insulin sensitivity (McMahon 1988). Plasma **glucagon** concentrations have been shown not to increase overnight and, in the absence of hypoglycaemia, plasma catecholamines do not reach levels at which they begin to exert significant metabolic effects (Campbell 1985a). The dawn phenomenon in Type 1 diabetic subjects has been shown to begin earlier (03.30am) and due to both an accelerated glucose production and impaired

utilization and not associated with cortisol, adrenaline or glucagon changes nor changes in insulin clearance (Campbell 1985b).

This leaves GH as the most likely candidate for the major role in the dawn phenomena. Early studies suggested that the alterations in glucose metabolism were initiated by the nocturnal surges in GH (Campbell 1985b) but these studies were carried out with the artificial pancreas (Biostater) and so doubt can be cast on their conclusions (Clemens 1982). However, when nocturnal GH was suppressed in adult diabetics using either somatostatin (De Feo 1986) or the anti-cholinergic drug methscopolamine (Davidson 1988), the dawn phenomenon was reduced by more than 70% or even abolished.

The dawn phenomenon may occur regardless of pubertal stage or glycaemic control in children and adolescents with Type 1 diabetes (Kobayashi 1997).

Further studies in elucidating the cause of the dawn phenomenon provide contradictory evidence regarding GH. In a small group of extremely well controlled adolescent diabetics Beaufrere and colleagues (1988) found no correlation between the extent of the dawn phenomenon and the nocturnal GH secretion. Similarly, in normal volunteers following octreotide administration with replacement of nocturnal rise of GH, no effects were seen on glucose, glucose production, disappearance or substrate oxidation following breakfast (Nielsen 1998). Contrary to this, in a large group of adolescents with average glycaemic control, overnight GH parameters were correlated with raised increased insulin requirements and higher blood glucose values in the early morning (Edge 1990a). However, the mechanism has not been clarified. Although **non-esterified fatty acids** are thought not involved to be in the dawn phenomenon (Boyle 1992), some studies show that NEFA have suppressive effects on peripheral glucose metabolism by inhibiting peripheral glucose oxidation and glycolysis (Bonadonna 1989, Walker 1991).

1.1.3 GH and lipolysis and ketogenesis

Clinical observations in normal subjects show that free fatty acids do rise in the night (Wildenhoff 1974) but the acute lipolytic action of GH is usually limited by a compensatory rise in insulin secretion. However, during chronic (24h) GH exposure, consistent lipolytic effects are seen (Metcalf 1981). Lipogenesis and lipolysis, which modulate fatty acid concentrations in plasma and tissues, are under hormonal control. In Type 1 diabetes, fasting NEFA levels are increased and there is both in vitro and in vivo evidence that GH helps to sustain nocturnal lipolysis in Type 1 diabetes. When compared to healthy control volunteers, Type 1 diabetic patients on their usual intensive insulin regime, have been shown to have a significantly exaggerated evening-night rise of NEFA (increased by three-fold) (Hagstrom-Toft 1997). This author found that nocturnal GH levels were also elevated in diabetic subjects and correlated to nocturnal lipolysis and adipose blood flow. Despite normal 24hour glucose levels, nocturnal rises in NEFA, GH and lipolysis were seen. Thus, nocturnal NEFA and lipolysis may be due to elevated GH but does not appear to be due to insulinopenia or hyperglycaemia.

Although circulating concentrations of **non-esterified fatty acids** (NEFA) are altered in intensively treated insulin-dependent diabetic subjects and a circadian change in NEFA concentrations has been noted, the mechanism of NEFA production directly by GH is still not clarified. When studying the **regulatory effects of GH** on lipid oxidation using specific bioassays to measure mitochondrial GH receptors in fibroblasts, GH was shown to directly stimulate fatty acid oxidation independent of insulin-like growth factor-I (Leung 1997). In addition, both preadipocytes and mature adipocytes do possess specific GH receptors. GH may therefore mediate both direct effects and act indirectly through the GH-mediated secretion of IGF-1 in adipose tissue. The prime mode of GH action in causing lipolysis has not yet been established in Type 1 diabetes.

In Type 1 diabetic subjects, the early-night peak of ketone concentrations have been associated with overnight GH release (Edge 1993) and the early-morning fasting ketone level is mainly determined by insulin levels. This ketogenic effect is also seen in Type 1 diabetic subjects who had euglycaemia, maintained with insulin infusion, were changed over to somatostatin and GH infusions and after only six hours on GH, when concentrations had reached those seen in poorly controlled subjects, plasma ketone increased markedly while blood glucose remained unchanged (Gerich 1976, Keller 1984). Hyperketonaemia may result from increased lipolysis (muscle and fat tissues) with increased free fatty acids supply for ketogenesis or from a primary action of GH on hepatic ketone body production or decreased peripheral utilisation. The primary mechanism is not clearly determined. Turnover studies performed in fasted and diabetic subjects suggest that a production rate of ketones just exceeding the clearance rate, causes a progressive build-up in ketone concentration by further antagonism of muscle clearance (Balasse 1989).

During fasting, both FFA and ketone bodies tend to suppress gluconeogenesis, but there is no consensus on the exact mechanism of ketone production and action (Fery 1996). Moller (1990) during his studies on forearm technique in Type 1 diabetic subjects, suggested that the main regulatory effect of isolated hyperketonaemia appeared to be a direct negative feedback inhibition of lipolysis. When comparing a 32hour short term fast in diabetic subjects and controls, the rate of development of ketosis, metabolic acidosis, NEFA and glycerol turnover increased progressively in both conditions, but the rate of rise of ketones was extremely accelerated when insulin deficiency occurred with fasting conditions (Burge 1993). However, these authors attributed elevated levels of glucagon and catecholamines on lipolysis as leading to the development of ketones. Further work reveals that following acute administration of GH in conscious dogs, while there was only a transient increase in NEFA at 20minutes, ketone body concentrations

were significantly elevated after 120-180 min independent of NEFA (Okuda 2001). In summary it is not clear whether in Type 1 diabetes the stimulation of lipid oxidation and ketogenesis is a continuous direct effect of GH or whether it is driven by increased substrate supply secondary to lipolysis.

1.1.4

SUMMARY AND HYPOTHESES

Although GH secretion is controlled primarily by GHRH and SRIH, the inter-relationship with other metabolic factors is complex. In particular, profound alterations in GH control are seen during fasting where a change in GH amplitude and pulsatility may mediate metabolic alterations, such as lipolysis and ketogenesis. The dissociation between GH and IGF-1 production suggests that the metabolic events in nutritional deprivation parallel that seen in Type 1 diabetes. This model can be used to explore various hypotheses of GH control and actions. At present it remains unknown whether the GH changes are primary or secondary and whether GH mediates metabolic effects directly or via mediators as the weight of current literature provides evidence to suggest either mechanism. In diabetic subjects, the raised GH levels and effects on nocturnal insulin resistance, NEFA and ketones can be separately evaluated by metabolic studies.

To clarify whether the changes in GH and metabolic alterations exist as specific physiological adaptations, such as seen in starvation or whether a pathological process continues to sustain a deteriorating scenario in the Type 1 diabetic subject, I have proposed a series of hypotheses to be tested by specific experimental conditions.

The three specific hypotheses that are suggested in this Thesis are based on the following

Observation

An altered pattern of raised GH in adolescents with Type 1 exists. The mechanism of raised GH requires exploration. Although GH clearance could differ to normal adolescents due be to alterations in GHBP, or glycaemic or insulinaemic states, the generation of the basic underlying pulsatile pattern of GH release may also be influenced by an imbalance of interactions between neurotransmitters and metabolites.

Hypothesis 1

GH hypersecretion exists in adolescents with Type 1 diabetes subjects and is a prime abnormality of GH control

Experimental Procedure

1. Examine GH clearance characteristics (half-life decay kinetics and distribution volume of exogenous GH) in Type 1 diabetic subjects and compare to reported control values.
2. Describe the characteristics of overnight GH secretion (mean, peak, pulsatility) by using mathematical methods comparing puberty-matched subjects (Type 1 diabetes and control groups).

Observation

Enhanced pulsatile GH secretion may result from alterations in SRIF, GHRH or cholinergic pathways or feedback of several potential metabolites on GHRH and somatostatin release. The direct role of insulin and glucose on GH control is still unclear.

The modulation of GH release by metabolic factors, particularly insulin and glucose, may have a greater regulatory role during fasting (nutrient-insufficient) state.

Hypothesis 2

Type 1 diabetes subjects have GH alterations similar to the physiological adaptation observed in prolonged calorie deprivation. GH pulse (peak and suppression levels) are influenced by insulin and glucose levels.

Experimental Procedure

1. Clarify the alterations of spontaneous overnight GH secretion and during euglycaemia or after altering cholinergic tone.
2. Identify the influence of insulin (eg hyperinsulinaemia) and glucose (eg hyperglycaemia) on endogenous GH suppression by using somatostatin. Assess the metabolic influence on GH rebound pulse secretion.

Observation

It is unclear as to whether GH mediates metabolic effects directly or via mediators to cause poor metabolic control, namely hyperglycaemia, insulin resistance, hyperketonemia and lipolysis. The components of the GH pulse that cause metabolic derangement remains unclarified.

Hypothesis 3

The importance of an abnormal GH signal is to directly cause metabolic deterioration in the Type 1 diabetic subject

Experimental Procedure

1. Reconstruct a GH pulse profile representative of the GH levels seen in adolescent diabetic subjects
2. Simulate such a physiological plasma GH profile under standardised conditions.
3. By reconstructing a plasma GH profile into its separate components (pulsatility versus continuous signal), the direct relationship of GH pulse characteristics on insulin resistance, lipolysis and ketogenesis can be compared during *in-vivo* study conditions.

In this Thesis, I examine GH kinetics, secretion and actions *in-vivo* by a series of studies described in detailed in Chapter 2 involving adolescents and young adults with Type 1 diabetes.

CHAPTER 2

EXPERIMENTAL PROCEDURES

2.0.0

PRINCIPLE METHODS

This Thesis reports on three experimental protocols that were devised in order to clarify questions in section 1.1.5.

Experiment 1 GH clearance study (subjects recruited by BRP)

Aims: Establish three metabolic conditions of glycaemia and insulinaemia (clamps) in order to examine

- a) Decay characteristics of GH (rhGH) half-life and distribution volume
- b) GH suppression during somatostatin infusion
- c) GH rebound secretion after somatostatin suppression

Experiment 2 GH secretion study (subjects recruited by Dr J Edge)

Aims: Analyse spontaneous overnight plasma GH profiles in adolescent subjects to perform

- a) Distribution analysis to calculate the mean, peak and trough plasma GH level representative of adolescents with Type 1 diabetes
- b) Deconvolution analysis to calculate secretory rates in Type 1 diabetes and control subjects during puberty
- c) Determine effects of euglycaemia on spontaneous GH secretion
- d) Effects of anticholinergic (pirenzepine) on GH secretion

Experiment 3 GH signal study (subjects recruited by BRP)

Aims: Examine the metabolic effects of the GH pulse by separating its components of pulse signal and continuous signal

- a) Effects on insulin sensitivity
- b) Changes representing the Dawn phenomenon
- c) Effects on lipolysis and ketogenesis

The recruitment of the patients, the general procedure for the studies and practical techniques are common to the different analyses in this thesis and to avoid repetition details are given in this chapter. In all studies using exogenous rhGH, Genotropin (Kabi, Stockholm) was used. The practical techniques, assay methods and analyses were the same in each of the studies and are provided in **Appendix 2 and 3**. Any specific methods or modifications are given in the relevant Chapter.

2.0.1 Subject recruitment & ethics

Three groups of subjects who were recruited from the Paediatric Diabetic Clinic and Young Adult Clinic at John Radcliffe Hospital, Oxford between the years 1988-1992. Patients recruited were those who had had a diagnosis of Type 1 (insulin-dependent) diabetes for a minimum of 2 years (to ensure endogenous insulin secretion was negligible) and were being treated with subcutaneous insulin and had no evidence of other medical diseases such as cystic fibrosis.

The Central Oxford Research Ethics Committee had approved all studies before recruitment began. Initial contact was at clinic attendance to explain the reason for carrying out the study and a standard letter was provided to eligible subjects and their respective parents to read. Thereafter, telephone contact was made 1-2 weeks later to discuss the information leaflet and any further clarification on practical procedures. All studies were carried out at the John Radcliffe Hospital, Oxford. Application and information letters were submitted to the Central Oxford Research Ethics Committee for ethical approval prior to each study protocol. Written information was provided to volunteers, their parents and to subjects' GP with further verbal discussions if subjects were interested in participating (see **Appendix Information: A1**). Written and verbal informed consent was obtained from subjects and guardian/parent with a 2-week period of reconsideration. As most patients needed to attend on at least three occasions, doubtful patients were not recruited. Later subject exclusion or withdrawal is detailed in **Appendix 2c**.

2.0.2

GH CLEARANCE STUDY

2.0.3 Study design

The secretion, half-rate of elimination and volume of distribution controls the concentration of a hormone in the circulation. Firstly, an assessment of the clearance of GH from the circulation is required.

The study design is illustrated in **Figure 2.1a**.

GH half-life was determined by (100mU rhGH) bolus after somatostatin suppression of endogenous GH in six patients each under three clamp conditions:

- (a) Normoglycaemia and normoinsulinaemia (NG)
- (b) Hyperglycaemia and normoinsulinaemia (HG)
- (c) Normoglycaemia and hyperinsulinaemia (IH)

Each subjects' clamp occurred in random order, separated by an interval of at least one week. In one patient (G) the normoglycaemic hyperinsulinaemic clamp was not performed. A computer programme was used to achieve stable clamping (**Appendix 5**). The different clamp conditions permitted analysis of the effects of short-term metabolic changes such as hyperinsulinaemia and hyperglycaemia on GH half-life.

This study protocol provided information on the effect of somatostatin on GH suppression under different insulinaemic and glycaemic states and allowed the analysis of GH rebound release after the termination of the SRIH infusion.

2.0.4 Subject characteristics (GH clearance study)

Six young adults (three females and three males *BCDEFG) median age 21.5 years (range 14.9 -25 years) with Type 1 (insulin-dependent) diabetes mellitus

Subject	A	B	C	D	E	F	G	Median
Age years	*	14.76	17.8	21	24	22.8	25	21.5
Sex	*	F	M	M	F	M	F	-
Weight kg	*	64.3	61.0	71.4	77.5	80.9	54.2	67.8
Height cm	*	162.3	167	174.2	172.5	169.5	166	168.5
Surface area m ²	*	1.7	1.68	1.85	1.92	1.94	1.58	1.77
BMI kg/m ²	*	24.4	21.8	23.6	26.0	28.3	19.6	22.6
HbA ₁ %	*	9.2	15.8	10.4	8.8	9.7	12.6	10.1
Duration Diabetes years	*	9.43	6.1	10	20	14	15	12
Insulin Dose u/kg/day	*	1.0	1.47	0.92	0.64	0.92	0.88	0.92

Table 1a Demographic data of subjects B-G attending Morning Studies

* Subject A was excluded from further analysis.

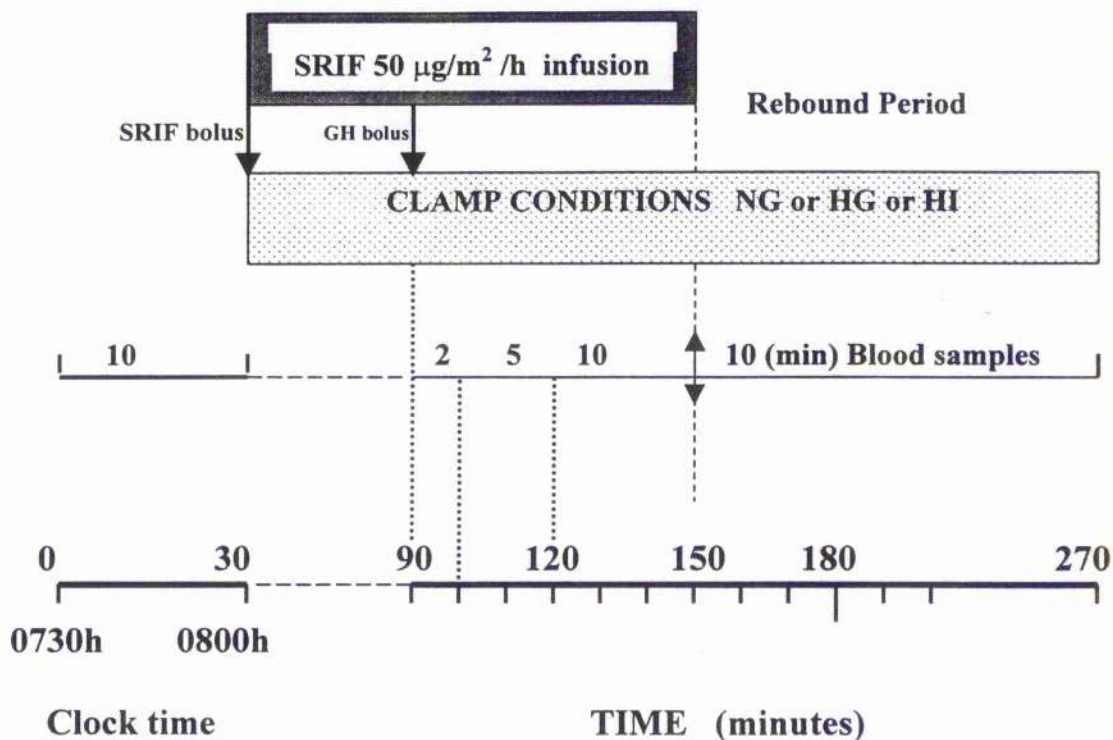


Figure 1a Study design: GH clearance

GH half-life: after withdrawal of long acting insulin at 36hours before study, subjects were admitted at 07.00h for cannulation.

Baseline samples were taken between $t=0-30\text{mins}$. Somatostatin (S) (bolus $50\mu\text{g}$ and infusion) was commenced at 08.00h and clamp conditions achieved $t=30-150\text{mins}$. Growth hormone(GH) 100mU was administered at $t=90$ and frequent blood samples taken to assess decay.

Clamp:

NG=normoglycaemic HG=hyperglycaemic, HI=hyperinsulinaemic

GH suppression: two periods $t60-90$ and $t120-150\text{mins}$ were analysed during 3 clamp conditions and somatostatin infusion.

GH rebound: at $t=150\text{mins}$ somatostatin was ceased; clamp conditions maintained until $t=270\text{mins}$ to assess GH rebound secretion.

were studied (Table 2.1a: Demographic data (B-G)). The median duration of disease was 12 years (range 6-20 years). Each diabetic patient received insulin (median 0.92 units per kilo per day, range 0.86 to 1.5) three to four times daily. They were on no other medication apart from insulin. All were in good health apart from their diabetes and there was no evidence of any long term diabetic complications in particular retinopathy or microalbuminuria as predictor of clinical diabetic nephropathy (Morgensen 1990). Based on arithmetic means of 5 to 10 glycosylated haemoglobin (HbA1) measurements over the previous two years in each patient, the mean HbA1 was 11% (range 9.2 - 15.6%; reference range 5.6 - 8.2%).

*Subject A was excluded as following this study various technical improvements were made.

2.0.5 Clinical Protocol

Preparation

All subjects had close telephone contact during withdrawal of long and medium acting insulin and an individual written regime was supplied to enable adequate glucose control with short acting (Actrapid) insulin.

GH clearance study illustrated in Figure 2.1a

Following an overnight fast and omitting evening insulin, subjects were admitted to hospital at 07.00 hours. An indwelling intravenous polyethylene cannula and a double lumen catheter were inserted under local anaesthetic (Figure A, Appendix 2) and subjects maintained in a resting position for 15 minutes. A continuous blood sampling withdrawal system was utilised (Figure B, Appendix 2). During the study no food was allowed but water was provided ad libitum.

Basal measurements for insulin, glucose and GH were started fifteen minutes after insertion of the cannula and thereafter at ten-minute intervals (07.30, 07.40, 07.50, and 08.00). The clamp was started at 08.00 hours

1. *GH suppression*: Endogenous GH secretion was suppressed by a *bolus* of somatostatin (SRIH) (somatostatin 1-14, 50 micrograms, Serono SpA, Italy) followed by an infusion of somatostatin (SRIH 50 $\mu\text{g}/\text{m}^2/\text{h}$)
2. *Clamp*: Insulin (Actrapid HM, Novo-Nordisk Gentofte, Denmark) was infused at a rate of 0.2 mU/kg. min to generate normoinsulinaemia with plasma insulin levels of 57-107 picomol/L (8 - 15 mU/L) or at a rate of 0.7 mU/kg/min to achieve elevated plasma levels of 360 picomol/L (50 mU/L). Blood glucose levels were maintained at 5 mmol/L for the normoglycaemic and 12 mmol/L for the hyperglycaemic clamp respectively. Having reached a stable clamp conditions, each clamp was maintained for at least two hours at the steady state for the duration of the whole study.
3. *Samples*: Initially blood glucose concentrations were measured at one to three minute intervals until a stable clamp was achieved.
Samples for insulin and GH were taken at ten to fifteen minute intervals throughout the whole study (Figure 2.1a).
4. *GH bolus half-life*: A bolus injection of the exogenous **monomeric** 22kDa recombinant human GH (*r*-hGH, 50 μg (100mU) Genotropin) was given at $t=90\text{mins}$. Following the *r*-hGH bolus, samples for GH were taken at two minute intervals for twenty minutes followed by two and three samples of five and ten minutes intervals respectively.
5. *GH suppression and rebound*: Examination of endogenous GH suppression by SRIH at $t=90\text{mins}$ and at $t=150\text{mins}$ under 3 clamp conditions is reported in Chapter 5. One hour after the recombinant GH bolus, the somatostatin infusion was terminated in order to analyse the endogenous GH rebound release (Chapter 5). Insulin, GH and glucose measurements were obtained at ten-minute intervals for the last two hours of the studies.

2.0.6 Experimental conditions

Clamp

Clamp conditions were established by 90mins and were maintained throughout the whole study period (90-270mins): mean \pm sem plasma glucose was 5.3 ± 0.1 , 5.3 ± 0.1 and 12.1 ± 0.1 mmol/L during normoglycaemic (NG) (n=6), hyperinsulinaemic (HI) (n=5) and hyperglycaemic (HG) (n=6) clamps respectively.

Study period (t90-150min) - Bolus half-life study

The insulin infusion during the normoinsulinaemic (0.2 mU/kg/min) and hyperinsulinaemic (0.7 mU/kg/min) clamp studies (t90-150min) induced mean \pm sem steady state plasma insulin concentrations of 36.4 ± 1.1 mU/l (NG) and (HG) and 92.2 ± 13.8 mU/l (HI) respectively.

Study period (t90-270min) - GH suppression and rebound

Mean \pm sem plasma insulin levels were NG 36.2 ± 6.2 and HG 30.9 ± 6 ; versus HI 77.52 ± 7.4 mU/L ($p < 0.001$) clamps respectively.

2.0.7

GH SECRETION STUDY- PROCEDURE 2

GH is secreted in a pulsatile fashion, so that multiple measurements over time during a 24-hour interval are required to assess endogenous release. However, daytime measurements require standardization of physical activities, meals and mobility and I have therefore analysed overnight plasma GH profiles. Evans (1987) concluded that in adults 15-20 minutes sampling was the lowest frequency that would identify all the major GH secretory episodes.

2.0.8 Study design

GH profile data were analysed in adolescents with Type 1 diabetes under 3 conditions and compared to controls. All subjects (diabetic and controls) had 12hour profiles from 20.00-0800h. The GH profile data have been the subject of a previous discussion (Edge 1990a) and the raw data re-analysed by Distribution Analysis.

2.0.9 Subject characteristics (GH secretion study)

Fifteen-minute integrated plasma GH samples were obtained from 29 diabetic adolescents (15 male, 14 female) and 34 healthy children (siblings: 17 male, 17 female) enrolled as control subjects in overnight studies (Table 2.1b). Adolescent diabetic subjects had had a diagnosis of Type 1 (insulin-dependent) diabetes for more than one year and were taking no other medication other than insulin. Normal adolescents who were healthy siblings of diabetic children were enrolled as control for overnight GH studies. All adolescents had height and weight measured for the study session and the height velocity was calculated over a six-month follow-up period. Pubertal stage was assessed by the method of Tanner 1962 (breast stage was used in girls and genital stage used in

Subjects Median values	Type 1 Diabetes	Control (normal healthy)
No	29	34
Age range years	13.6 10.5-16.5	13.1 9.1-20.1
Sex	15M 14F	17M 17F
BMI range kg/m²	19.1 15.8-23.9	18.9 14.5-26.3
Puberty Stage 1	6	9
2-3	12	13
4-5	11	12

Courtesy of J Edge

Table 1b Demographic data of diabetic and control adolescents attending for overnight (20.00-08.00h) plasma GH profiles in order to perform Deconvolution analysis to estimate secretory rates.

boys). Skeletal age was assessed in diabetics as part of the annual review of growth and development.

The two groups were comparable in age (median age: diabetics 13.6y, range 10.5-16.5; controls 13.1y, range 9.1-20.9y), stage of puberty (as assessed by method of Tanner 1962) and weight (median Body Mass Index: diabetics 19.1, range 15.8-23.9; controls 18.9, range 14.5-26.3). Diabetic subjects had a median glycated haemoglobin (HbA_{1c}) concentration of 10.6% (range 8.4-19.5) and had had Type 1 diabetes for more than 1.5 years (median 6.1y, range 1.5-12.8). They were on no medication apart from insulin. All control subjects had normal HbA_{1c} concentrations (Median 6.0%, range 4.2-7.8).

2.1.0 Clinical protocol

GH profiles for estimation of secretion

Exogenous insulin was discontinued before admission for overnight studies: ultralente at least 44 hours, lente 36 hours, soluble insulin 8 hours before the study night. The subjects were admitted at 1730h and had a small low-fibre meal (2/3 of normal carbohydrate intake) before 1800h and then fasted during the study. Venous cannulation and methods were as described previously. Overnight glucose clamping was carried out from 20.00-08.00h and the target blood glucose entered into the clamp programme was 5.0mmol/L.

All subjects (29 Type 1 diabetes, 34 control) had 12hour overnight GH profiles from 20.00h-08.00h (15-minute integrated sampling). The control subjects were admitted on a single night: the diabetic subjects were admitted on two occasions and were studied under different conditions. The raw data of 15minute plasma GH profiles obtained from diabetic NR, EC and PIR were analysed by the Distribution Method (Chapter 6) and then subjected to deconvolution analysis.

2.1.1 Experimental conditions

Normal insulin regime group (NR)

Fifteen (7M, 8F) adolescents with Type 1 diabetes were maintained on their usual insulin regime and had their normal meal and snack pattern with the last snack taken before 22.00h.

Euglycaemic Clamp group (EC)

Twenty-nine (15M, 14F) adolescents with Type 1 diabetes (including 15 subjects studied during normal insulin regime night) underwent an overnight euglycaemic clamp protocol after exogenous insulin had been discontinued 36h before admission.

A variable rate insulin infusion was used to achieve and maintain euglycaemia (target blood glucose 5mmol) between 20.00-08.00h based on 15min bedside glucose measurements with rate of insulin infusion being calculated by a microcomputer programme (Matthews 1989, Appendix 5). Blood glucose concentrations below 6.0 mmol/L were achieved by 21.30h. Overnight (01.00-08.00h) mean \pm SD plasma glucose was 5.26 ± 0.04 mmol/L. The principle of clamp programme is provided in Appendix A5.1.

Pirenzepine group (PIR)

Seven of the adolescents who were clamped on the first night underwent a second identical overnight euglycaemic clamp but with the addition of two 50mg oral doses of the anticholinergic drug pirenzepine given at 19.45h and 22-23.00h to suppress their endogenous GH secretion (Martina 1987, Page 1987a).

The plasma glucose concentrations were mean \pm SD 5.36 ± 0.14 mmol/L between 01.00-08.00h. Results of deconvolution analysis under the three experimental conditions are provided in Chapter 4.

2.1.2

GH SIGNAL STUDY

Studies examining the metabolic effects of GH in-vivo are complex, particularly as the human model is subject to individual variation. Changing metabolism occurs on a daily, hourly or minute basis and interferes with both inter-subject and intra-subject study variability. In order to standardise daily routine, I have examined subjects overnight both to minimise exercise activities and meal-cued events. Sleep is also a normal activity contributing to the daily metabolic timetable of Type 1 diabetic subjects and observation overnight should be of immense interest.

2.1.3 Study design

The Distribution analysis performed on plasma GH profiles is an objective method of estimating baseline, mean and peak hormone concentrations (section 4.0.7) and allows representation of the spectrum of GH levels experienced by adolescent diabetics.

GH is unstable when given as an infusion unless a buffer solution is used. The GH infusion rates would also depend on individual clearance of the hormone and in order to construct a standard GH profile in all study subjects, various adjustments and metabolic parameters need attention. Diabetic subjects also require standard metabolic conditions of glucose and adequate insulin throughout any study period.

Standardised, reproducible double-blind study conditions should be achieved in order to differentiate the GH signal and its effects on metabolites, particularly when examining ketogenesis, lipolysis and insulin sensitivity.

Aims

- 1) Achieve adequate endogenous GH suppression overnight (12 hours)
- 2) Standard metabolic conditions of glucose and insulin
- 3) Prevent hypoglycaemia or exaggerated fall in glucose stimulating counter-regulation
- 4) Reproduce a physiological GH profile seen in adolescents with Type 1 diabetes, separating components of the GH message
- 5) Achieve standard reproducible control conditions for each study night
- 6) Investigator and subject to be blinded to the GH infusions given
- 7) Adequate observation time to examine changes in insulin, glucose, ketones and lipolysis

2.1.4 Subject characteristics (GH signal studies)

Eight subjects (HJKLMNPQ) with Type 1 diabetes were initially recruited and their individual details are given in Table 2.1c. Subject exclusion and refusal (detailed in Appendix 2b).

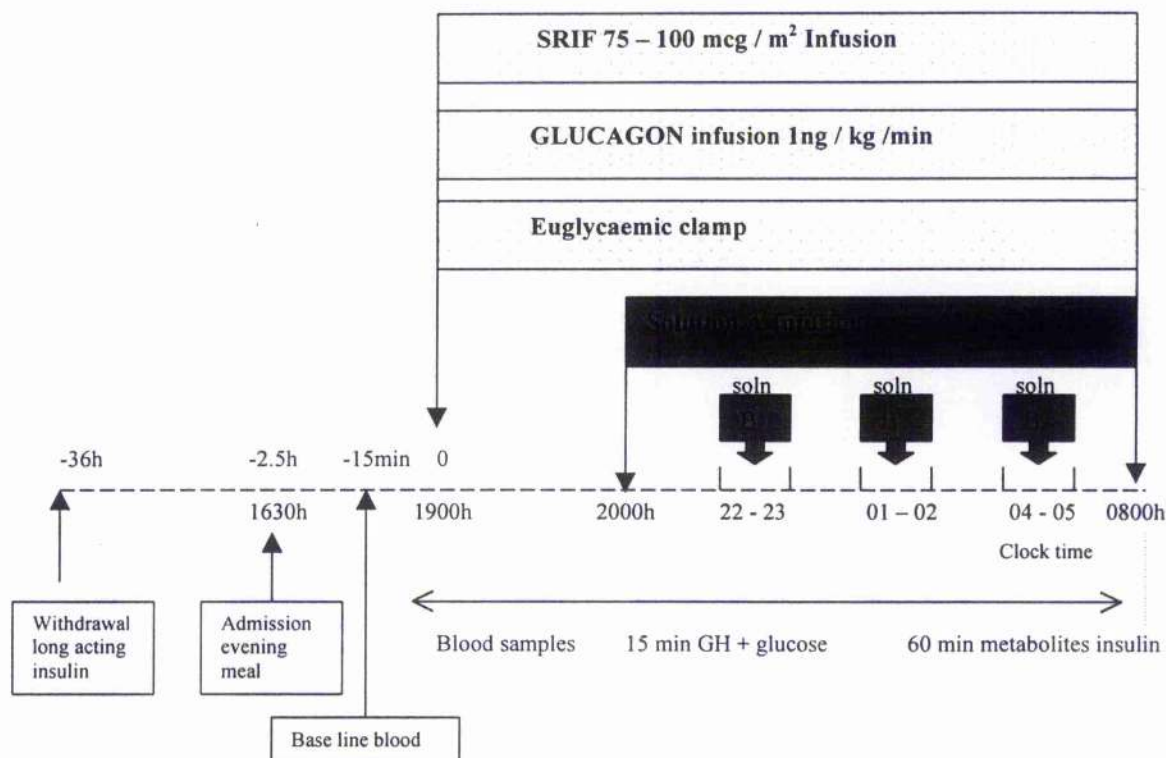
Metabolic and hormone data were analysed from six subjects who were young adults (2 female, 4 male JLMNPQ), median age: 19 years (range 14-26), median weight was 66.6kg (range 52-80.75), median surface area 1.78 m² (range 1.48-2.05) and they were non-obese (median BMI 23.6, range 21.9-26.9 kg/m²). Median duration of diabetes was 10 years (range 6-18). Each was controlled on insulin (median 0.68 U/kg/day, range 0.45-0.95) given 2-4 times daily. Median glycosylated haemoglobin (HbA_{1c}) level taken nearest the study dates of 8.8% (range 6-12.5%; reference range 3.8-6.0%).

All subjects (H-Q) were on no other medications apart from insulin. All were in good health except for their diabetes and there was no evidence of any long term diabetic complications. Based on two nearest HbA_{1c} to study dates, mean HbA_{1c} was 8.9% (range 6-14, reference range 5.6 - 8.2%); group median height 165cm (range 154.1 to 189cm), the median surface area 1.84 m² (range 1.4 - 2.05m²) and the median BMI 21.9 kg/m² (range 17.3 - 27.9kg/m²).

Subject	H*	J	K*	L	M	N	P	Q
Age years	13	14	17	27.9	16	22	26	19
Sex	F	F	M	M	F	M	M	M
Weight kg	44	65	66.6	78.6	52	74.4	80.8	58.1
Height cm	159.9	162.3	174	167.8	154	169.5	189.0	160
Surface area m ²	1.41	1.68	1.78	1.91	1.48	1.94	2.07	1.6
BMI kg/m ²	17.3	20	22	24.4	21.9	26.9	22.6	23.6
HbA _{1c} %	8.9	7	12.5	11.5	8.8	9.7	6.6	9.6
Duration Diabetes years	4	7	8	18	6	14	10	15
Insulin Dose u/kg/day	1.09	1.16	0.78	0.87	1.08	0.92	0.77	0.65

Table 1c Demographic data of all subjects (H–Q) recruited for overnight metabolic studies

Of subjects who had GH signal studies, two were excluded* before reporting metabolic data, but their GH data was utilised to assess GH half-life (HJKLMP 60minute pulse; JLMNPQ 12h infusion) (Chapter 2).



Key

Profile night	Solution A	Solution B
Pulse	Buffer	r-hGH
Continuous	r-hGH	Buffer
Control Reference	Buffer	Buffer

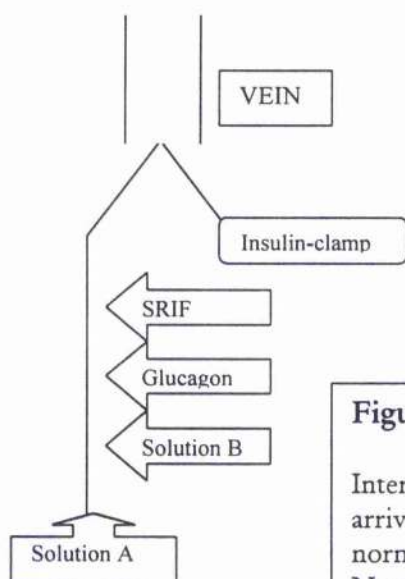


Figure 1b Study design: GH signal

Intermediate acting insulin was stopped thirty-six hours before arrival at the hospital. Each patient was studied overnight under normoglycaemic conditions by a variable rate insulin clamp. Normoglycaemia was achieved by fifteen-minute bedside glucose measurements with an iterative computer programme to predict insulin requirements.

2.1.5 Clinical protocol (GH signal study)

Each subject was admitted on 3 separate nights (19.00-08.00h). Five (JLMPQ) out of the 6 subjects participated on all 3 nights; one (N) for pulse GH and control. The study was double blind to both operator (BRP) and patient and the sequence of these studies was varied at random 2-6 weeks apart. Subjects were admitted at 16.30h on the study day and ate before 18.00h.

The schematic diagram of the study design is shown in **Figure 2.1b**. Exogenous insulin was discontinued at least thirty-six hours before the study. A small easily absorbable carbohydrate meal, being two-thirds of daily carbohydrate intake, was given before 18.00 hours with a continuous intravenous infusion of soluble insulin to limit the blood glucose rise. Subjects then fasted overnight during the study 1900-0800h.

Standard conditions

In order to observe the direct metabolic effects of GH, standard conditions were maintained: reproducible GH signal, insulin-varying clamp, somatostatin infusion and glucagon infusion. Overnight infusions were administered as shown in the inset of schematic diagram in **Figure 2.1b**.

2.1.6 "Convolution" of GH signal

From the results in Chapter 4, adolescent subjects with Type 1 diabetes have overnight plasma GH represented by

"Continuous GH Signal"

at OC50 level of $6.14 - 8.38 \text{ mU/L} = \text{average of } 7.4 \text{ mU/L}$

"Pulse GH signal"

at OC75 and OC95 of $24.99 - 52.71 \text{ mU/L} = \text{average is } 39 \text{ mU/L}$

A GH profile to mimic these levels was thus reconstructed. The components of the GH signal, the pulse and continuous GH signal was separately examined and compared to a control night without GH.

The calculations were based on GH distribution volume in diabetic subjects obtained from bolus GH decay (section 3.0.4 (6.1L, 8.9% body weight) and assuming steady state is reached after 5 half-lives.

Calculation

Continuous Infusion dose mU/kg/h

$$\begin{aligned} \text{dose of GH} &= \text{desired steady state plasma level} \times \text{DV} \times \lambda \times 5 / 8.9 \\ &= 7.4 \times 6.1 \times 0.693 \times 5 / 8.9 = 17.5 \text{ mU/kg total dose} \\ &= 1.5 \text{ mU/kg/h over 12 hours} \end{aligned}$$

Pulse infusion mU/kg/pulse

$$\begin{aligned} \text{Total dose GH} &= \text{desired plasma level} \times \text{DV} \times \lambda \text{ (half-life decay constant)} \\ &= 39 \times 6.1 \times 0.693 / 8.9 = 18.5 \text{ mU/kg total dose} \\ &= 6 \text{ mU/kg/pulse as 3 pulses} \end{aligned}$$

Total dose of 18 mU/kg r-hGH either as solution A or B was provided by independent operator (Pharmacy, John Radcliffe Hospital).

Growth hormone solution (80mU/ml) was made up as follows: 4U r-hGH (Genotropin 4iu, Kabi, Stockholm, Sweden) was diluted in 50ml phosphate buffer solution under aseptic conditions.

Operator Blinded to GH signal

The GH/buffer or buffer only was provided to the operator (BRP) in 50ml syringe labelled as solution A and solution B.

On all study nights both solution A and B were administered as either

Solution A: 12h continuous intravenous GH infusion to achieve a raised baseline mean level (1.5mU/kg/h given between 20.00-08.00h) (where Solution B=Buffer)

Solution A was infused at 0.02 ml/kg/hr (20.00-08.00h) using Vickers syringe pump and the same solution and syringe was used.

or Solution B: Three separate GH infusions given hourly (6mU/kg/h at 22.00-23.00h, 01.00-02.00h, 04.00h-05.00h) to reproduce the pattern of nocturnal pulses of GH (where Solution A=buffer)

Solution B was infused at 0.08ml/kg/h at 3 separate hourly periods.

This syringe was infused by modified Vickers pump attached in series circuit to a multi-function digital timer* (setting accuracy: $\pm 0.5\%$ of set time; repeat accuracy: $\pm 0.3\%$ of set time) and this was programmed for time setting at 60min on/120min off to control infusion rate. The clock was set to start at 20.00h in "off" phase.

or a control night: where both solution A and B contains buffer* solution (i.e. no replacement of GH).

The difference in magnitude of GH pulses and continuous signal was 3.4 mimicking the difference seen in diabetic subjects (Chapter 6) .

A Buffer* solution alone was used for control nights. The code was announced after all study nights were completed, but before analysis of data was carried out.

*Appendix information: A2 – multifunction digital timer

2.1.7 Standard conditions of glucose

Overnight glucose clamp was carried out from 19.00h to 08.00h. A microcomputer programme using portable bedside Apple IIe computer (Appendix 5, A5.1 and A5.2) calculated the rate of the insulin infusion. The target blood glucose concentration entered into the programme for the clamp studies was 5.0mmol/L. During the clamp, the mean insulin infusion rate for

the attainment of a steady blood glucose concentration was used to calculate insulin requirement for the maintenance of euglycaemia.

Insulin: The insulin infusion (0.1 U/ml) was made by adding 5U of soluble insulin (Actrapid, Novo, Copenhagen, Denmark) and 2ml of subject's blood, to prevent adsorption (Kerchner 1980), to 48ml 0.9% saline. The syringe was changed three times during the night and new insulin was primed in the syringe before use on each occasion. The insulin was infused using a Treonic IP3 Syringe pump (Vickers medical, Basingstoke, U.K.). An overnight euglycaemic clamp was undertaken in all subjects.

Glucose clamp conditions

Euglycaemia (<6 mmol/l) was achieved by 22.00h on all study nights and lowest blood glucose measured at any time was 4.0 mmol/L. During control nights ($n=6$, SRIH alone) blood glucose fell below 3.5mmol/L in 5 subjects and <3.0 mmol/L in 3 subjects within the first 2 hours. When additional GH was administered with SRIH ($n=10$), a blood glucose below 3 mmol/L was only measured during one study night and below 3.5mmol/L in five nights. No subject needed correction of low blood glucose. A stable euglycaemic clamp was achieved after 3 hours in all subjects. Euglycaemic mean clamp levels achieved on study nights (01.00-08.00h) were comparable: 5.5 ± 0.5 during continuous GH, 5.9 ± 0.5 during pulse GH vs. control 5.3 ± 0.4 mmol/L. Infusions of glucagon and somatostatin were both commenced at 1900h and interactions with the insulin clamp are discussed below and expanded in Chapter 5.

2.1.8 Endogenous GH suppression

Somatostatin: Endogenous GH was suppressed by somatostatin throughout the study period. The Somatostatin infusion (30 μ g/ml) was made by adding 1500mcg of a 3mg vial of somatostatin 1-14 (Stilamin, Serono,S.p.A, Rome, Italy) dissolved in 2ml water, to 49ml 0.9% saline and infused at a rate of 50-

100 mcg/m²/h between 19.00-08.00h by a similar Vickers syringe pump. The solution was changed after 6 hours.

Endogenous plasma GH levels were effectively suppressed within one hour of SRIH infusion on all study nights (1.4 ± 0.4 continuous GH, 0.8 ± 0.2 pulse GH, 1.1 ± 0.3 control mU/L) before administration of GH/Buffer.

Glucagon: Because of the prolonged SRIH suppression, glucagon infusion at 1 ng/kg/min (Novo-Nordisk, Gentofte, Denmark) was added to the clamp and maintained throughout the study period in order to achieve constant hepatic glucose production. The glucagon infusion (1mcg/ml) was 0.5mg of 1mg vial of glucagon (Novo Industri, Bagsvaerd, Denmark) in 10ml 0.9% saline (50mcg/ml) then adding 1ml of this to 49ml 0.9% saline. The same solution and glucagon was used throughout the study and the glucagon was infused through a Vickers syringe pump at a rate of 1ng/kg/min between 19.00-08.00h.

2.1.9 Samples

The blood was collected in 15min aliquots in lithium-heparin tubes for later GH assay but the first 2 or 3 drops of blood for each tube were collected in fluoride tubes for immediate blood glucose assay. Samples from an individual session (involving 3 bolus or GH infusions (60min or steady state) were assayed together after completion of their respective study sessions.

Hourly samples were also collected in lithium-heparin tubes for plasma free insulin, metabolites and non-esterified free fatty acids (FFA) assays.

Samples for GH, blood glucose, insulin were analysed as described in **Appendix 3d**. Samples for GH analysis were spun, separated and stored at -20°C until assay. Validation of GH assays between reporters were also clarified (**Appendix 3e**).

Convolution of GH plasma profile and the metabolic effects of these profiles is the subject of discussion in Chapter 6.

2.2.0

JUSTIFICATION OF PRACTICAL PROCEDURES

The complexity of metabolic control, particularly during the non-steady state of normal life in subjects with Type 1 diabetes, makes interpretation of probable metabolic processes from single observations impossible. In order to gain insight into the possible disordered metabolism, prevent the least disruption to normal routine and obtain standardised conditions, I have studied most of my subjects during the overnight fasted state. The sleeping, fasted state also constitutes a regular part of normal life experience and facilitates multiple observations of key metabolites (metabolic profile). However, this sort of study will in practice generate a very large number of samples.

2.2.1 Blood sampling

Continuous integrated blood sampling was performed in all studies unless otherwise stated. An indwelling intravenous polyethylene cannula and a double lumen catheter were retrogradly inserted under local anaesthetic and cannulation is described in **Figure A, Appendix 2**.

The blood was collected in fifteen minute aliquots into tubes into a fraction collector (L.K.B. Fraction Collector, L.K.B. Instruments Ltd., Surrey) for later assay but the first two to three drops of blood for each tube was collected in tube caps. This blood was assayed at the bedside for glucose using a Yellow Springs whole blood analyser (Y.S.I. model, Clandon Scientific Ltd., Aldershot, Hants). Details of sampling frequency for GH and metabolites, assay procedures and handling of samples are given in **Appendix 2.0, 3.0** and discussed in respective Chapters.

2.2.2 Use of insulin-varying clamp

It is impossible to quantify a requirement for the complete normalisation of metabolism in Type 1 diabetes since even with the strictest control of blood glucose, blood ketone levels and other intermediate metabolites often remain persistently elevated above normal. Therefore, the term "insulin requirement" is generally interpreted as meaning the quantity of insulin necessary to maintain normoglycaemia. Also insulin requirement will vary according to dietary intake and degree of physical activity and in order to eliminate these variables, basal (i.e. post-prandial) and resting insulin requirements are probably the most useful measure.

Current insulin doses are often equated with insulin requirement, but for several reasons this is an inaccurate estimate. Even when glycaemic control appears good there is a high incidence of unrecognised hypoglycaemia, particularly at night (Whincup 1987) confirming that insulin requirement is not equal to the insulin dose regimes. The insulin requirement to maintain euglycaemia has been calculated by other investigators to be 7-20 mU/kg/h using a variety of methods (Clarke 1980). Waldhausl (1982) calculated the basal insulin production in healthy man as being 15 ± 6 mU/kg/h and such an infusion rate markedly improved metabolic control.

A device that measures the blood glucose, and then administers only sufficient insulin to maintain normal glycaemia would effectively quantify insulin requirements. Such close linked methods of blood glucose control, where insulin is given in direct response to a measured concentration of blood glucose, have been used therapeutically in brittle diabetics and those undergoing surgery and are the basis of a sliding scale of the insulin dose.

I have used an insulin-varying clamp that predicts insulin requirements accurately, without giving rise to pulsatile insulin infusions or the need for glucose infusions to maintain euglycaemia. Thus the effects on insulin requirements during GH administration can be clearly observed. The glucose

clamp by insulin-varying method used in this Thesis is described fully in Appendix 5.

The programme developed by Matthews, 1989, works on the principle that it progressively assembles a data array from its own evolving experience "of that particular individual and then uses the array to choose the next insulin infusion rate, thus making no assumptions and introducing no bias. This system has been shown to be sensitive and the insulin infusion rate is equal to the insulin requirement for the maintenance of euglycaemia. The computer programme developed by Matthews, 1989 allows adjustment of insulin in order to maintain euglycaemia (or any other pre-determined glucose concentration). Because insulin takes a finite time to act, the adjustment takes place only every 10-15 minutes; a total time-period of several hours is thus needed for such clamping. The programme does not use any fixed algorithm to predict insulin requirements, but uses instead a principle of experience rather than preconception as the basis of the prediction (Matthews 1989). Observer bias is thus eliminated in the results. This technique therefore yields a robust estimate of overnight insulin requirement in adolescent diabetics and is able to provide additional information concerning the insulin-glucose interactions.

2.2.3 Use of genotropin

Biosynthetic GH (Somatorm, Pharmacia) was the first available for routine prescription in 1985. Somatorm consists of the same 191 aminoacids present in endogenous GH plus an additional methionine that is present as a consequence of the biosynthesis (risk of denaturing the molecule if removed). Despite this Somatorm is physiologically indistinguishable from pituitary GH (Milner 1985).

Advances in recombinant DNA technology have allowed the biosynthesis of Genotropin that is structurally identical to endogenous GH. Genotropin is produced in *E.coli* modified genetically to produce human GH, which is

harvested from the bacteria after a period of fermentation. The hormone then undergoes a series of purification steps and is freeze-dried. (Fryklund 1987)

This production process (Fryklund 1987) results in an extremely pure hormone identical to endogenous GH and with full biological activity.

2.2.4 GH profiles

Until it was recognised that GH secretion was pulsatile early measurements which were of single serum GH levels yielded uninterpretable results. It was later felt that since the hormone appeared to be released in an apparently random fashion only a mean integrated concentration could be of any value.

Studies involving GH profiles require frequent and prolonged sampling periods and the interpretation of GH pulsatile changes during puberty, when a multitude of dynamic endocrine interactions and physiological changes are occurring, is often difficult and confusing.

Before the pulsatile pattern of the hormone profile can be examined it must be certain that the timing and frequency of blood sampling is adequate to answer the questions asked. It should be decided whether continuous or discreet sampling is required: continuous integrated sampling is less likely to miss peaks or troughs and a pulse will always be included, although its amplitude may be slightly underestimated (Matthews 1988). Albertsson-Wikland & Roseberg showed that during twenty-four hour GH profiles of four children, integrated and discreet sampling produced almost identical profiles and there was no obvious blunting or widening of peaks with the integrated sampling technique. Sampling should be sufficiently frequent not to miss any pulses and in practice should include at least five samples per oscillatory cycle (Matthews 1988). However, there is evidence that more intensive (five minute) sampling of GH will unmask higher frequency pulses although there was no pulse identification at this frequency that was not contiguous with the majority of the major of the episodes which were all uncovered at less frequent sampling intervals (Evans 1987). The same study concluded that in adults fifteen to twenty minutes

sampling is the lowest frequency that will identify all the major secretory episodes. These findings have also been substantiated in studies by Edge 1990 of children during puberty.

2.2.5 Endogenous GH

Heterogeneity of peptide hormones in biological fluids is both a general phenomenon and a relative one. For endogenous GH, heterogeneity in serum arises from 3 sources: in the secreting organ or tissue; in the circulation and peripheral tissues; and during handling procedures in the laboratory. The pattern of GH heterogeneity will always differ between the calibrant and the patient specimens and the heterogeneity will differ between subjects and within a subject under differing circumstances. However these conclusions on GH heterogeneity are based upon a number of complex physicochemical tools of high selectivity, not upon immunoassays. For the most part antibodies are blind to these GH variations and heterogeneity of GH proteins is only one of several factors contributing to inter-laboratory dispersion of GH immunoassay results (Seth 1988a, Seth & Hanning 1988b). These problems have been well reviewed by Baumann (1990) and Smith & Norman (1990). From a combination of these sources and mechanisms, although GH in serum is highly heterogeneous and previous assays of GH may reflect a sum of many different components, specific immunoassays which detects 22kD fragment has been used in this Thesis.

Protein binding may or may not affect the physiological and clinical significance of GH estimates. Since in-vivo clearance is affected by protein binding, concentrations in plasma at any one time will not necessarily reflect secretion rate; plasma will become selectively enriched with slowly cleared fractions. GH has a number of epitopes (antibody-binding domains) which can be mapped using mono-epitopes specific monoclonal antibodies. The GH epitopes are thought to be still exposed when protein bound and this is the main reason why protein binding does not appear to have a major effect on

immunoassay results. In addition, binding proteins appear not to be able to compete with antibodies for available GH (Baumann 1987). While this is of interest and possible physiological relevance, there is no evidence that it alters the clinical significance or interpretation of GH assay results (Baumann 1990).

2.2.6 Use of somatostatin

Somatostatin, a gut and brain peptide is a powerful inhibitor of *in vivo* and *in vitro* GH release and inhibits the GH response to all known stimuli (Wass 1983). In man somatostatin blocks the GH response to exercise, arginine and insulin hypoglycaemia. The dose of SRIH required to achieve adequate GH suppression has been examined and a dose of 50-100µg/h was utilised in previous studies with adequate endogenous GH suppression (Skamene 1984). Native somatostatin, a 14-residue peptide, because of its short circulating half-life, must be given by continuous intravenous infusion. Somatostatin (Stilamin, Serono) is a synthetic tetradecapeptide hormone (SS-14) with several physiological actions including inhibitory effects at the level of the hypothalamus. It is presented in a freeze-dried sterile powder form and for infusion purposes it can be diluted to the required volume with physiological saline and protected from light. The continuous intravenous infusion is stable for up to 72 hours at room temperature.

2.2.7 Use of glucagon

Subject H undergoing 12-hour somatostatin without glucagon was excluded from analysis, as she became profoundly hypoglycaemic and this may have initiated adrenergic responses. In previous studies in which iv bolus of SRIH was given alone or short acting insulin was given alone, or with somatostatin, SRIH enhanced the hypoglycaemic action of insulin and delayed the restitution of blood glucose to normal (Gerich 1976). This influence of SRIH was ascribed to hypoglucagonaemia since it could be eliminated by replacement doses of glucagon and not GH (Rizza 1979).

In order to prevent hypoglycaemic-induced counter-regulation, a glucagon infusion was maintained during GH signal studies. The dose of glucagon infused at 1ng/kg/min was used in my studies as this dose has been shown to achieve plasma levels of around 75-100 pg/ml (Paolisso 1987), similar to the physiological basal glucagon levels of 100-150 pg/ml (Kuku 1976).

Basal glucagon seems to be responsible for at least one third of glucose output. Basal circulating glucagon concentrations in insulin-dependent diabetics have been reported to be normal (Unger 1970) or increased (Gerich 1975b) compared to normal subjects, but are always inappropriately high for the degree of glycaemia. In diabetes, the glucagon predominance may contribute to the increased net hepatic glucose output and elevates blood glucose values (Gerich 1975b). Increased lipolysis, mainly due to insulin deficiency, elevates fatty acid supply to the liver, which responds to the hyperglucagonaemia with increased fatty acid oxidation and ketogenesis (McGarry 1979). This concept has also been disputed because of the apparent transient effect of experimental hyperglucagonaemia on hepatic glucose production (Felig 1976, Sherwin 1976a). In the study reported by Paolisso, 1986, somatostatin was used to inhibit endogenous pancreatic hormone secretion, glucagon was replaced by continuous glucagon infusion and insulin was administered in a pulsatile or continuous manner. The doses of insulin did not completely inhibit hepatic glucose production and plasma insulin averaged 7 mU/L during continuous infusion and oscillated between 1.5 and 35 mU/l during pulsatile delivery. Glucagon replacement here resulted in peripheral plasma glucagon levels averaging 180-200 pg/ml (versus basal values 110-120 pg/ml) and the possibility existed that this over-replacement of glucagon may have stimulated hepatic glucose production too strongly to be affected by the peripheral plasma insulin achieved. Paolisso, 1987 investigated more precisely the role of glucagon in modulating the effects of insulin delivery. At low glucagon levels (75-130pg/ml) the superior pulsatile effect of insulin became manifest after approximately the third hour of insulin infusion but this critically depends on circulating glucagon

levels, being completely abolished if plasma glucagon levels are in the range of 200 pg/ml (Paolisso 1987). The doses of glucagon used in my studies should not antagonise insulin effects on liver carbohydrate metabolism (Paolisso 1987). Furthermore, glucagon does not influence lipolysis in normal subjects when infusing glucagon at rates between 0.5-1.5 ng/kg/min (achieving maximum levels 110-130 mg/L) (Gravholt 2001). These observations suggest that the glucagon infusion chosen for my studies would not influence glucose disposal, insulin action or stimulate lipolysis.

In summary, when achieving standard conditions, adequate GH suppression can be achieved with somatostatin infusion but counter-regulation of exaggerated hypoglycaemic effects can be minimised with glucagon infusion at low levels (75-130pg/ml), thus enabling accurate observations of glucose/insulin changes due to GH alone.

Statistical Analyses

Full details of statistical analyses are provided in Appendix 4 and the main statistical authorities used are provided in the Bibliography.

Specific statistical analyses used, results and findings are described separately in each chapter.

General Principles of statistical analysis used in this Thesis

Standard formulae were used for the calculation of means, standard deviations (SD) and standard error of the mean (SEM).

Group results of metabolic data are given as mean \pm SEM unless otherwise stated. Where SD are provided this can be converted to SEM =SD/ \sqrt{N} , where N is number of subjects in study. Parametric statistics are used to describe statistically significant differences eg Student's paired t-test was used for comparisons of mean data between subjects' overnight profiles and at individual time periods; unpaired t test

Subject demographic data are described as median \pm range and non-parametric statistics applied eg Mann-Whitney U, Spearman rank correlation) were used as appropriate.

Data Handling

Normalising data (section A4.2)

Blood glucose and insulin infusion data were normally distributed.

Log-transformation was used to normalise the ketone, lipid and plasma free insulin data. Thus, parametric statistical techniques have been applied on these log-transformed data.

Missing Data (section A4.4)

This amounted to only 0.3% of the metabolic and hormone data and was due to either insufficient sampling or a laboratory analysis problem.

Plotting and Graphical representation

The results are plotted graphically by computer programmes updated through Microsoft Office programmes.

Programmes used for Statistical analysis (section A4.3)

Between 1989-1993, most of the calculations were done on the Oxford University IBM computer with some later usage on the Birmingham University Honeywell DPS-8/70M mainframe computer.

The programmes used were OXSTAT ver 4 and 5 and SLIDEWRITE and the BMDP Biomedical Computer Programs (University of California, Berkley, USA). Post 1993, Microsoft Office programmes performed word-processing, data transfer and analysis and Stats view and Minitab programmes (including the online help facility in Minitab 10.51).

Statistical analyses applied

(i) Students' *t* test

Students' *t* test comparing means at each time point may show statistically significant differences at specific time points but not show whether there was an overall difference between the profiles, although if the majority of individual time points were different then the whole profile would probably be different. Also the biological significance of a statistical difference at one or two time points during a 12hour-study night is difficult to assess.

Thus a total mean value for a period studied eg overnight can be calculated for each patient in a group and these values then compared between groups as non-paired *t*-test.

(ii) ANOVA, Analysis of Variance (one way and two way)

Analysis of variance (2-way ANOVA) was used to examine changes with time and confirm differences between study nights.

Two-way analysis of variance with metabolite concentration classified by group and time (Armitage, Blackwell Science,UK). It is possible with this method to compare the differences in the variance between two groups removing the variance due to the time the samples were taken. Each sample value is used in this method rather than a derived mean. The significance of the difference between groups was calculated from the variance ratio using F-tables.

In chapter 6, two-way Anova on log-transformed ketone data was used to confirm change with time (20.00-08.00h) and when plateau levels were reached ($p=NS$).

Similarly, steady state GH was determined by ANOVA when serial serum GH concentrations over a 12hour period showed no significant drift (section 3.0.5).

(iii) Mann-Whitney test

The Mann-Whitney test was used to compare the GH secretion data between puberty groups as this was not normally distributed. The Mann-Whitney test is commonly regarded as a test of medians where one median is an estimate that of the probability that one variable is less than the other (Altman 1991). However, it is a test of both location and shape. Given two independent samples it tests whether one variable tends to have values higher than the other. However, it is also important to look at distributional differences or spread. I have therefore provided the range of GH secretion for each puberty group. This will provide the features that are most clinically important. The hypothesis that there was no difference between puberty groups can be rejected as both median and spread of the difference are different between each puberty group.

(iii) Regression and Correlation

Regression functions can be either linear or of higher form. By changing the x or y scale non-linear functions are always converted, to linear ones, since the mathematical treatment for this is simpler ($y=a+bx$). This

relates to the common regression of y to x , where y is the dependent variable and x the independent variable.

The opposite regression, x to y , gives as a rule a regression line somewhat differently inclined to that of the regression y to x . The measure of the mutual behaviour of these two lines gives the correlation coefficient r .

The square of the correlation coefficient r^2 is the measure of certainty and shows to what extent the independent variable x influences the dependent variable. A measure of certainty of 0.5 indicates that 50% of the changes in y can be explained as due to changes in x . There is no actual proof that the connection is a causal one and the result should be regarded as a pointer to the direction in which further investigation would yield direct proof.

(iv) Confidence Intervals

The confidence interval is the range on either side of a sample mean. The 95% confidence level (α of 0.05) was used. When measuring plasma GH at very low levels, the GH suppression level may be influenced by the limits of the GH assay (eg sensitivity of assay 0.3 mU/L and intra-assay CV at GH concentration of 2.9mU/L is 8.0%). The confidence interval may allow a better description of reliable these suppression limits. The confidence interval (CI) may be viewed as the range of possible values for the true difference that are statistically likely around the point estimate (mean) values obtained in each clamp ie above or below 95% CI would be a probability of seeing a difference of $p < 2 \times 0.025$ (Gardner & Altman 1986).

Specific Mathematical Analyses used

Chapter 3 (section 3.0.1- 6) specific mathematical formulae were used to calculate

Monoexponential Decay of r -hGH

Biexponential Decay Model

Volume of Distribution

Steady State Half-Life

The metabolic clearance rate (MCR) of r -hGH

Chapter 4

Matthews (1991) has described using a method of cumulative distribution and probability to provide an objective assessment of GH profiles (section 4.0.1).

Probit Transformation

Continuous summation (from left to right) of the area under the normal distribution and plotting the areas against a linear abscissa yields a *sigmoid curve*. The values of its ordinates corresponds to the areas of the normal distribution. The sigmoid curve may be transformed into a straight line by means of the probit transformation, whereby the frequency percentages (=areas x100) are converted into corresponding deviations of the normal distribution increased by the addition of 5 and these are known as *probits*. Probits may be obtained without the need for calculation from tables (Statistical Tables published by Oliver and Boyd, Edinburgh).

Deconvolution analysis (section 4.0.2).

Several methods of deconvolution have been proposed (detailed in Appendix 1b). I have used the Deconvolution model proposed by Hindmarsh and its advantages are discussed in section 4.0.3.

GH profile data was provided to DRM and mathematical calculations made by computer programmes to calculate to GH secretion. The mean overnight secretion (and range) by puberty group is described in Chapter 4. GH secretion data is described by standard summary statistics.

Time Series analysis (section 4.0.4)

Fourier analysis was performed on GH secretory data. The calculations were made by Oxford modelling software. The Fourier transform of the GH secretory data demonstrated the dominant pulse periodicity and revealed any differences in puberty groups, during euglucaemia and after pirenzepine.

Chapter 5

Clamp analysis are detailed in section 5.0.1.

Calculation of clamp bias, insulin clearance and coefficient of variation have been made.

Chapter 6

Cross-correlation

Cross-correlation is an iterative technique for establishing whether there are statistically coincident recurring waveforms (of any shape) within a data array (Matthews 1983). One data array is serially correlated against the other with progressive step changes in the time-relationship between the data. The result is dependent both on the relative amplitude of such waves or pulses (eg whether large pulses of one array are associated with large pulses of the other) and on the phase relationships between the arrays (whether one data array is time-lagged with respect to the other. It is independent of absolute concentrations of hormone.

Correlation regression analysis was performed to examine the relationship between GH parameters (log10 suppression or peak and mean rebound) and concurrent, previous hourly and mean plasma free insulin and glucose levels for an appropriate time period. Spearman's rank correlation was used to measure the degree of association between GH parameters and subjects' characteristics.

CHAPTER 3

DISTURBANCES OF GH CLEARANCE

3.0.0

INTRODUCTION

Elevated hormone levels may result from abnormalities in continued GH secretion or decreased metabolic clearance. Previous pulse detection programmes and time series analysis does not provide adequate information on actual secretory events nor account for possible variations in metabolic clearance rates. Deconvolution analysis models using an iterative method of "curve stripping" would be required to address the particular issue of increased GH secretion. Deconvolution analysis requires assumptions or measurement of GH disappearance rate and volume of distribution but these parameters have not been clarified in Type 1 diabetes. Other factors such as glucose concentrations or insulin levels may also affect half-life and volume of distribution in subjects with Type 1 diabetes.

In this Chapter the half-life analysis of exogenous GH (*r*-hGH), after endogenous GH suppression, was determined in young adults with Type 1 diabetes after bolus GH (GH clearance study); after 60minute and steady state infusion of *r*-hGH (GH signal study). The experimental conditions allowed the examination of GH clearance, GH suppression and GH rebound release during metabolic conditions of normoglycaemia, hyperglycaemia and hyperinsulinaemia.

Control Subjects for bolus and GH pulse infusion are detailed in Appendix 2. Group demographic data confirms that subject characteristics of these control subjects were similar to bolus GH clearance study (Table 3.2a).

Subjects Median values	Type 1 Diabetes	Control (normal healthy)	
		Hindmarsh 1989/90	Shah 1999
No	6 (B-G)	10	19
Age range years	21.5 14.9-25	19 17-45	27 22-36
Sex	3M : 3F	10M	13M : 6F
Weight range kg	67.8 54.2-80.9	68.1 46-82.5	-
Height range metres	1.690 1.62-1.74	1.695 1.63-1.74	-
Surface area range m ²	1.77 1.18-1.92	1.79 1.53-1.99	1.8 1.23-2.4
BMI range kg/m ²	22.6 16-25.9	21.2 17-25.4	22.5 16-31

Table 2a Summary of demographic data comparing diabetic subjects (B-G) with reported controls.

3.0.1 Specific mathematical analyses

Data are given as mean \pm SEM. The half-life of GH was calculated from log_e transform of its plasma concentration. The relationship between time and the transformed data was described by linear regression. Curve fitting was performed with the BMDP statistical software package (University of California). The plasma GH concentrations at entry ($t=0$) in each of the studies were compared using two-way analysis of variance (ANOVA) on a repeated measures design (Snedecor and Cochran, 1980). Two-tailed paired student's *t*-test was used to compare GH half-life under three different clamp conditions and also to compare the distribution volume (DV). The statistical significance was set at $p < 0.05$.

The relationships between the GH half-life and plasma GH concentration were analysed by regression of an orthogonal, second-degree polynomial, with statistical confidence intervals determined from the joint parameter variance. Simple linear regression and Pearson's correlation coefficient were used to evaluate a priori null hypothesis of no relationship between GH half-life, volume of distribution and prevailing glycaemic and insulin levels.

Comparison of group data from control and diabetic subjects were made via unpaired two-tailed Student's *t* statistics, assuming unequal variances.

3.0.2 Monoexponential decay

A monoexponential equation was used to fit the plasma GH concentration decay curves, with statistical confidence intervals calculated for the individual GH half-life estimate after each bolus injection. Half-life ($t_{1/2}$) was calculated from the Ln-transformed plasma GH concentrations using a mono-exponential model

$$[GH] = A_0 (e^{-\lambda t}) + B$$

A_0 is the amplitude of the phase after the distribution of the hormone in the sampling compartment and as previously shown the distribution of

the hormone injected is complete after six minutes (Matthews 1985). The distribution phase was derived from each individual concentration-time plot as the point where the observed data deviated from the extrapolated decay curve. Therefore the six minutes values were the start point taken for the mono exponential analysis.

- λ is the elimination rate constant and identical to the slope of the linear regression.
- B is basal serum GH concentration just before the r-hGH bolus injection and therefore approaches zero when endogenous GH secretion is suppressed by SRIH infusion.

The relationship between time and the transformed GH concentrations was described by linear regression. As the half-life is the time taken for a 50% reduction concentration it can be calculated by dividing $\ln 2$ (0.693) by the slope (λ) of the regression line).

3.0.3 Biexponential decay model

In order to compare this mono-exponential model with the bi-exponential model as described by Faria, (1989), data were fitted into the equation:

$$[GH] = A_0 e^{-(\lambda_a t)} + B_0 e^{-(\lambda_b t)} + C$$

- A_0 is the amplitude of the a-phase, corresponding to the rapid distribution of the hormone in the sampling compartment.
- B_0 is the amplitude of the b-phase, corresponding to the decay of GH from the circulation.
- C is the basal plasma GH concentration just before the r-hGH bolus injection and therefore approaches zero when endogenous GH secretion is suppressed by SRIH infusion.

Lambda (λ_a and λ_b) are the elimination rate constant ($t_{1/2} = \ln 2$) and identical to the slopes of the linear regressions. The mean parameter values were estimated by non-linear, weighted, least squares modified Gauss-Newton iterative analysis with the subsequent calculation of asymmetric joint confidence limits for the

precision of fit for all parameters considered simultaneously (Johnson 1983). F ratio testing demonstrated that a biexponential decay model was not justifiable statistically at $p < 0.01$ (protected α for multiple comparisons).

3.0.4 Distribution Volume

The distribution volume (DV, litres) was calculated by dividing the total amount of GH injected (dose, mU) divided by the peak plasma GH concentration achieved at the time $t=0$ min. The plasma concentration of GH (mU/L) at $t=0$ min was calculated from the intercept (A to regression).

$$DV = r\text{-hGH dose injected (100 mU) divided by A}$$

3.0.5 Steady State half-life

Steady state was determined by ANOVA of the serial serum GH concentrations over the 12 hours showing no significant drift. This inference was confirmed by linear regression defining a zero slope.

At steady state, the continuous IV GH infusion rate equals the product of the measured plasma GH concentration (mean over last 6 hours after reaching steady state levels) and the rate constant of elimination (k) and the distribution volume (DV) (Schaefer 1996)

$$\text{GH infusion dose} = [\text{plasma GH steady state}] \cdot k$$

The half-life at steady state is determined from $t_{1/2} = \ln 2/k$

The metabolic clearance rate (MCR) of *r*-hGH was also calculated for subjects who received a constant infusion of GH (IHJLMQ). This was calculated by method of Rosenbaum 1989:

$$\begin{aligned} \text{MCR} &= \frac{\text{infusion rate of continuous GH}}{\text{Steady state plasma GH concentration}} \\ \text{MCR (L/min)} &= k \cdot (\text{DV diabetic subjects}) \end{aligned}$$

3.0.6 GH Rebound Half-life

Calculation of the precise half-life of GH after discontinuation of somatostatin (rebound/release of endogenous GH) was difficult when GH remained elevated since it is impossible to exclude ongoing GH secretion. The time between the start of the rise of GH plasma concentration and the time when the peak plasma GH concentration was reached were calculated. In those that had a single peak ($n=9$ clamps), with the assumption that that additional GH secretion was negligible, the half-life was estimated. The characteristics of the plasma GH rebound is illustrated and discussed further in Chapter 4.

3.0.7

RESULTS

3.0.8 GH clearance: bolus

Details of clamp conditions are provided in Table 3.2b and metabolites are discussed in Chapter 4.

Growth hormone

Figure 3.2a demonstrates the GH profiles achieved during each clamp condition. The GH rebound and suppression data discussed later.

Mean GH concentrations after suppression of endogenous GH with SRIH were 1.5 ± 0.4 mU/L ($n:17$), mean peak levels reached after 100mU bolus injection were 17 mU/L (range 13-21.8 mU/L). The peak levels obtained were similar under the three clamp conditions (t90-150mins Table 3.2c).

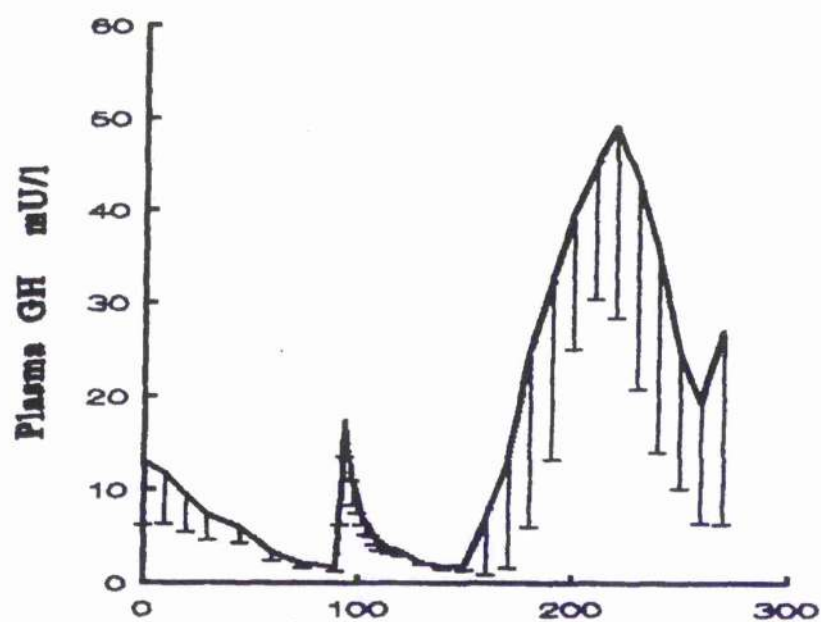
Half-life: Monoexponential model

The distribution phase was rapid and complete by 6 minutes as has been demonstrated in previous studies of normal subjects (Matthews 1985, Hindmarsh 1989). The disappearance of exogenous γ -hGH described by a

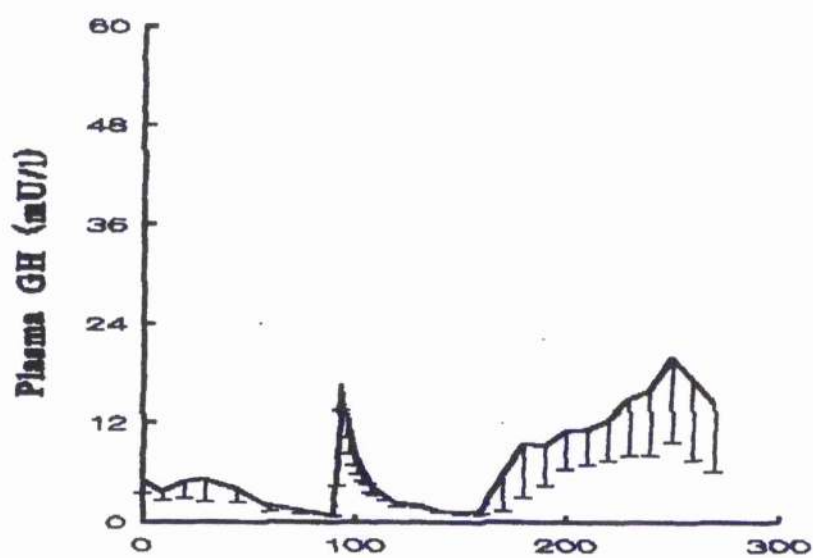
Clamp Conditions by study time	Normoglycaemic N=6	Hyperglycaemic N=6	Hyperinsulinaemic N=5
Time 90-150			
Plasma Glucose Mean (SEM) mmol/L	5.4 \pm 0.1	12.1 \pm 0.3	5.5 \pm 0.2
Plasma Insulin Mean (SEM) mU/L	48.0 \pm 7.3	34.7 \pm 6.6	92.2 \pm 13.8
Time 150-270			
Plasma Glucose Mean (SEM) mmol/L	5.2 \pm 0.04	12.07 \pm 0.02	5.2 \pm 0.06
Plasma Insulin Mean (SEM) mU/L	29.9 \pm 5.2	28.9 \pm 5.9	69.9 \pm 7.9
Time 90-270			
Plasma Glucose Mean (SEM) mmol/L	5.3 \pm 0.1	12.1 \pm 0.3	5.3 \pm 0.1
Plasma Insulin Mean (SEM) mU/L	36.2 \pm 6.2	30.9 \pm 0.6	77.5 \pm 7.4

Table 32b Clamp conditions achieved for morning studies when GH half-life (t90-150), GH suppression (t90-150) and endogenous rebound examinations (t150-270) were performed.

Clamps: NG=normoglycaemic HG=hyperglycaemic HI=hyperinsulinaemic.



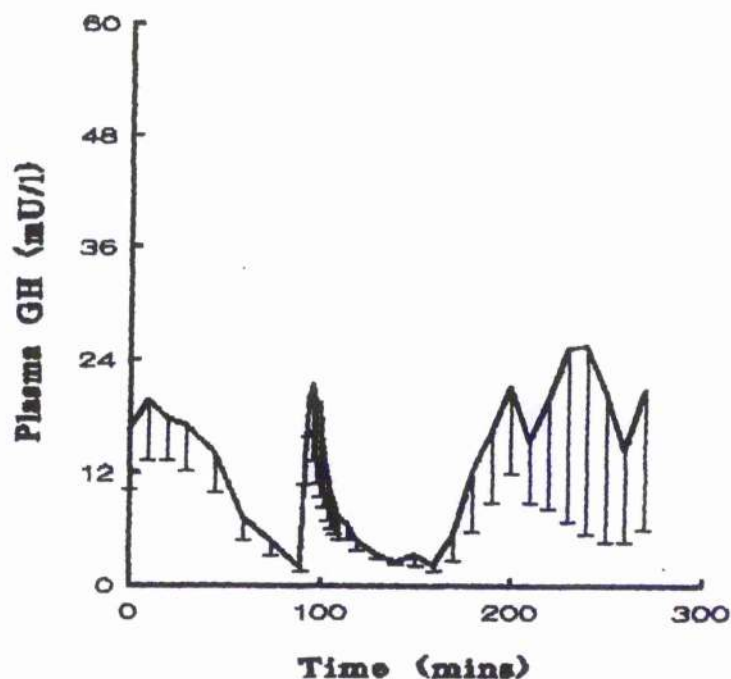
Normoglycaemic clamp conditions



Hyperglycaemic clamp conditions

Figure 3:2a

Continued.....



Hyperinsulinaemic clamp conditions

Figure 3.2 a GH profiles during GH clearance study (t0-270mins)

Plasma GH levels during three clamp conditions assigned in random order

GH half-life (t90-150mins) After bolus dose of 100mU GH at t90mins, the GH decay showed no difference between the three clamp conditions.

GH rebound after withdrawal of somatostatin infusion at t=150mins shows wide individual variation (note wide SEM bars) during all clamps.

There was no relationship between endogenous rebound GH levels and prevailing glucose or insulin levels.

mono-exponential model was calculated after the distribution phase was complete.

Figure 3.2b illustrates the Ln-transformed plasma GH concentrations following a bolus GH injection in two patients under three clamp conditions and shows the linear regression line of the phase corresponding in general to irreversible metabolic loss of GH from the circulation. Linear regression analysis of the loge transformed GH concentrations revealed a mean correlation coefficient (r-value) of 0.96 (SD 0.03, n=17). Calculated mono-exponential GH disappearance curves after distribution was complete and the mean regression line describing the disappearance of the exogenous GH under the three clamp conditions was obtained for all subjects. Data on the half-life, volume of distribution, intercept (mU/L) under the three clamp conditions are summarised in **Table 3.2c**. Mean half-life during clamp : NG 14.2 (range 10.6-21); HG 11.1 (range 9.5-13.1); HI 15.9 (range 10.9-27.7). There was a large variation within each patient under the different clamp conditions but the differences did not reach statistical significance. There was no trend in the data to suggest that either the distribution volume or the half-life was consistently altered by the clamp conditions. Therefore the data of the 3 clamp conditions were pooled. After suppression of endogenous GH with SRIH and following a bolus injection of 100mU r-hGH

GH half-life after bolus injection was

mean \pm SEM 13.6 \pm 1.9 min (Range 11.9-19.4 min, n:17)

The half-life was significantly prolonged in the diabetic subjects (Student's t test $p < 0.05$) compared to the control group of Hindmarsh, 1989 (mean 8.9 min, Range 6.3 -11.6) (Appendix 2).

Half-life biexponential model

Table 3.2d shows the half-life of GH of the B-phase corresponding in general to irreversible loss of GH from the circulation estimated by bi-exponential

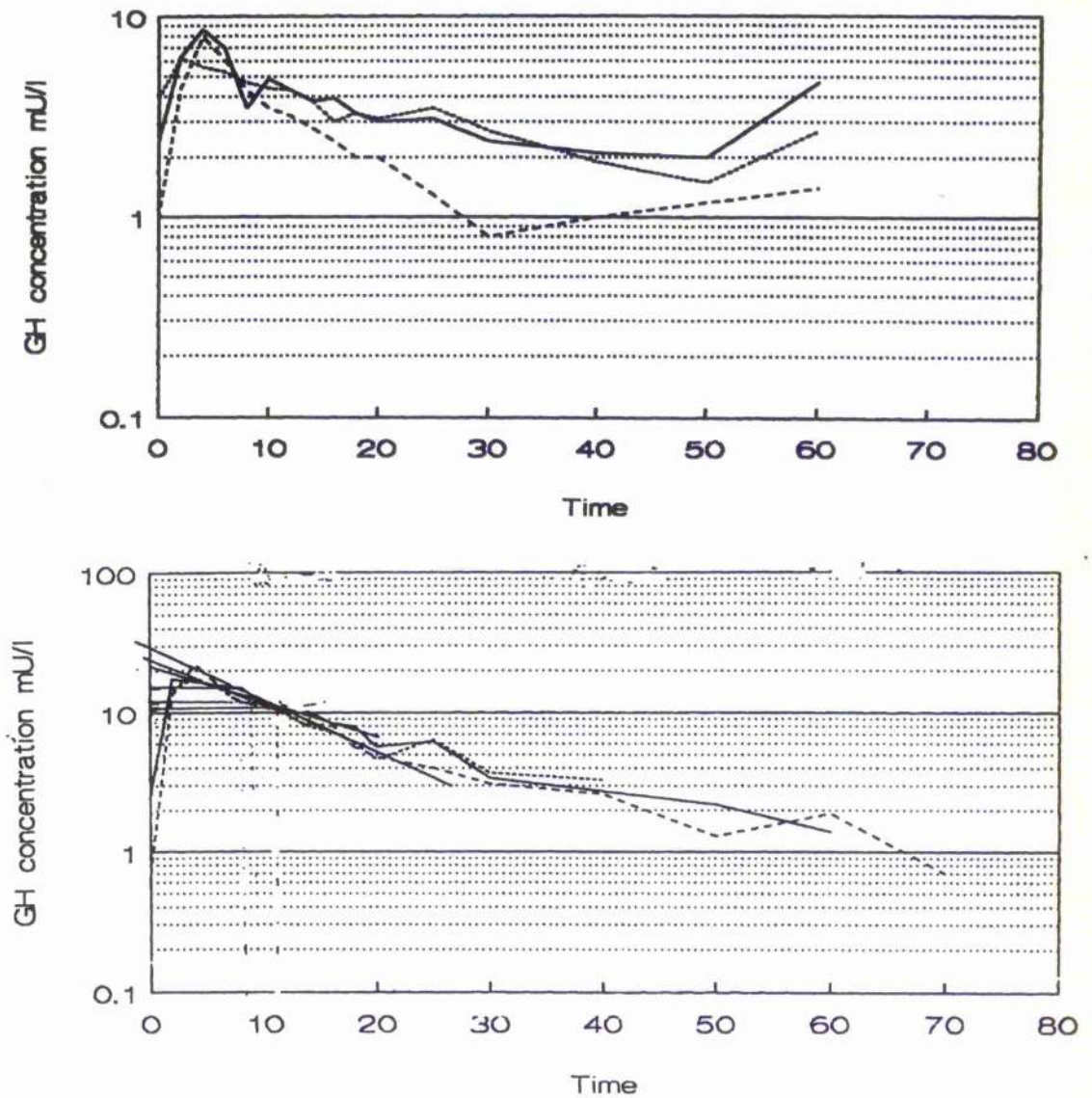


Figure 3.2b Decay characteristics after GH bolus

\ln transformation of plasma GH before calculation of monoexponential decay.

Upper panel: plasma GH decay over time (minutes) during 3 clamp conditions.

This subject illustrates variable decay curves.

Lower panel: Manual calculation of GH decay curve. This subject shows no difference in GH decay during 3 clamp conditions

Subject	B	C	D	E	F	G	Mean (SEM)
Clamp NG							
Intercept mU/L	14.2	12.6	23.2	25.4	12.2	21.2	18.2 (1.2)
Distribution volume L	7.0	7.9	4.3	3.95	8.2	4.7	6.0 (0.8)
GH half-life min	17.3	21	10.6	12	12.1	12.5	14.3 (1.6)
Clamp HG							
Intercept mU/L	12	15.4	18.5	21.6	13.5	23.6	17.5 (1.0)
Distribution volume L	8.3	6.5	5.4	4.6	7.4	4.2	6.1 (0.7)
GH half-life min	9.8	9.5	11.8	10.7	13.1	11.8	11.1 (0.5)
Clamp NG							
Intercept mU/L	18.5	11.2	20.8	18.2	15.1	--	16.7 (0.8)
Distribution volume L	5.5	8.9	4.8	5.5	6.63	--	6.3 (0.7)
GH half-life min	10.9	27.7	16.1	13.1	11.8	--	15.9 (3.1)
Group mean (SEM)							
Intercept mU/L	14.8 (0.7)	13.2 (0.6)	20.8 (0.7)	21.6 (1.1)	13.6 (0.4)	22.5 (0.6)	17.5 (0.6)
Distribution volume L	6.9 (0.8)	7.8 (0.7)	4.8 (0.3)	4.7 ± 0.4	7.4 (0.4)	4.5 (0.3)	6.1 (0.4)
GH half-life min	12.7 (2.3)	19.4 (5.3)	12.8 (1.7)	11.9 (0.7)	12.3 (0.4)	12.2 (0.4)	13.7 (1.3)

Table 3.2C Monoexponential calculation of bolus GH half-life and distribution volume.

Data are given for each subject (B-G): Results following *r*-hGH 100mU bolus under 3 clamp conditions. There was no statistical difference between the three clamp conditions and therefore mean of all clamps are pooled.

Clamps: NG=normoglycaemic, normoinsulinaemic HG= hyperglycaemic, normoinsulinaemic HI= normoglycaemic, hyperinsulinaemic

Subject	B	C	D	E	F	G	Mean (SEM)
Clamp NG							
GH half-life min CI	18.1 17.3-18.9	19.8 19.0-20.6	11.2 10.8-11.6	11.7 10.9-12.6	12.8 12.0-13.6	13.1 12.4-13.8	14.5 1.47
Clamp HG							
GH half-life min CI	9.6 9.1-10.1	10.2 9.4-11.0	11.5 10.9-12.1	11.5 10.9-12.1	13.4 11.8-15.0	11.4 9.2-13.2	11.3 0.54
Clamp HI							
GH half-life min CI	11.3 10.7-11.9	26.6 24.4-28.6	17.3 16.5-18.1	14.3 13.8-14.9	12.3 11.8-12.8	--	16.3 2.74
Subject mean CI							
GH half-life min	13.0 12.3-13.6	18.9 17.6-20.1	13.3 12.7-13.9	12.5 11.9-13.2	12.8 11.8-13.8	12.3 10.8-13.2	14.0 1.6

Table 3 2dB exponential equation suggests there was no significant difference from monoexponential fit for GH half-life.

CI contains the 67% joint statistical confidence limits for the precision of fit.

Clamps: NG = normoglycaemic HG= hyperglycaemic HI= hyperinsulinaemic

analysis, in which the first component (α -phase) was 3.1 ± 0.6 (SD) min. The pooled overall mean was 13.9 minutes (median 12.3, Range 9.6-16.7, n:17). The bi-exponential analysis did not show a significantly improved fit of the disappearance curves allowing for change in degrees in freedom and the estimated half-lives were not significantly different (Student's paired t-test: df 16, $t = -1.39$, $p = 0.183$).

Distribution Volume (DV)

The mean \pm SEM volume of distribution in **diabetic patients** was 6.1 ± 0.4 L (range 4.45-7.8, n=17 clamp studies) compared with the mean value in the control group of Hindmarsh 1989 (3.54 ± 0.32 L, n=14) and Shah 1999 (2.67 ± 0.28 L). (DV in diabetic subjects 0.089L/kg vs. control 0.054L/kg).

3.0.9 GH clearance: 60min pulse infusion

Mean plasma GH concentration after suppression of endogenous GH with SRIH was 3 mU/L (SD 2.6); mean peak plasma GH level achieved during 60 minutes GH pulse infusions was 33 mU/L (SD 6.4).

Half-life monoexponential model

Three 60-minute GH infusions were applied in each patient at 2-hourly intervals and the half-life data of the GH infusion study was estimated using the monoexponential model equation (Table 3.2e). No significant improvement on the squared-sum deviation was gained by the use of more complex exponential equations. Excluding 2 out-liers, results are

Pulse	Mean GH half-life mins	SEM	p value
First pulse	27.2	3.3	NS (1 v 2)
Second	26.5	2.8	NS (2 v 3)
Third	23.9	1.6	NS (3 v 1)

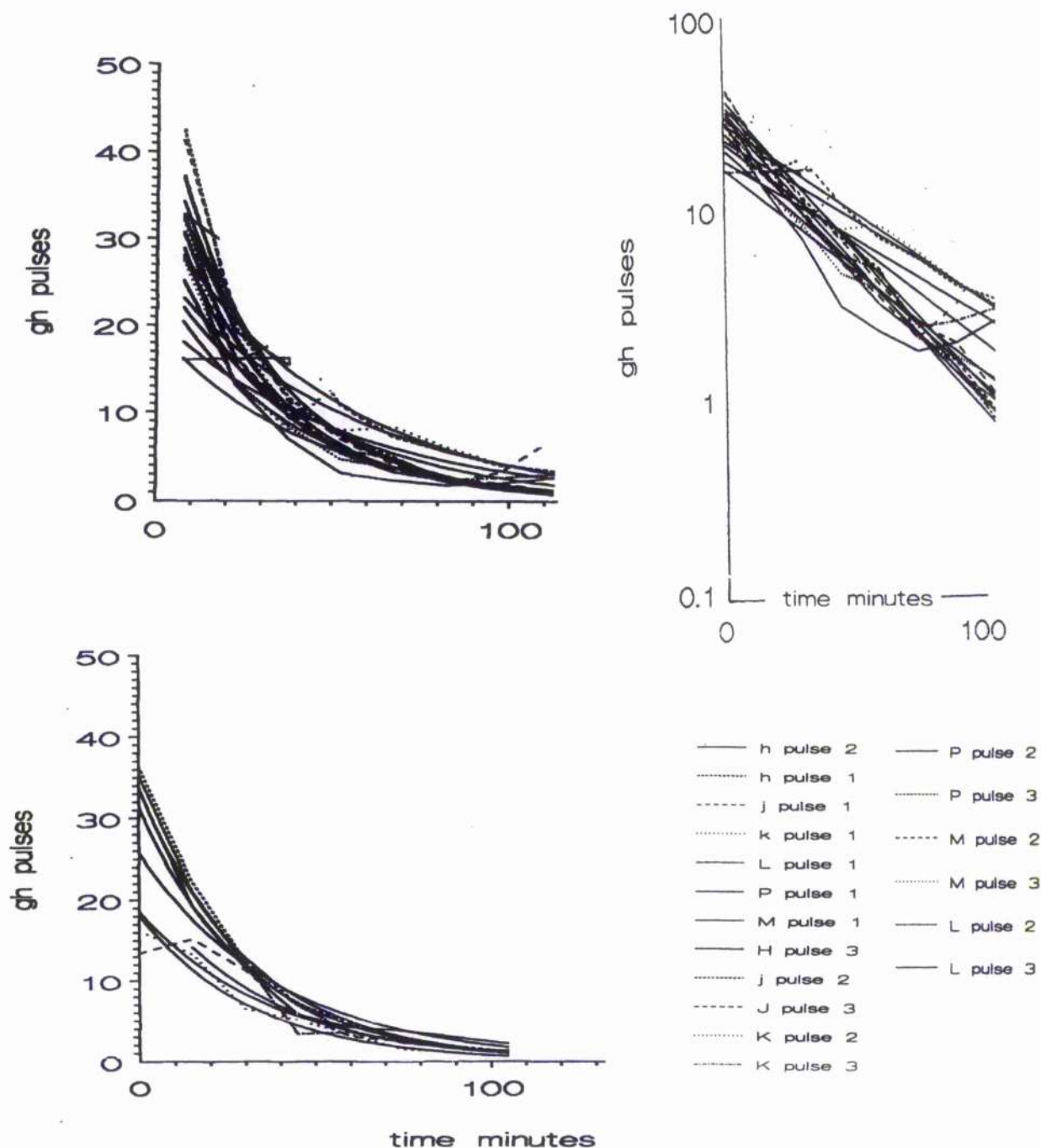


Figure 2c Decay Characteristics after 60 minute GH pulse infusion

Plasma GH concentrations mU/L (y axis) were calculated $y = Ae^{-\lambda x}$ for each 60 minute pulse decay over the following 0-100mins (x axis). Comparing three pulses (1,2,3) for each subject, the mean exponent λ was 0.028. There was no association of GH half-life with peak plasma GH concentration.

Ln transformation of GH concentrations are also shown (right panel). DV was calculated by another method as distribution is unpredictable during prolonged GH infusion.

	H	J	K	L	M	P	Mean (SEM)
Total dose injected 6mU/kg per pulse mU	264	390	339.6	471.6	312	484.8	377
PULSE 1							
Exponent	-0.0211	-0.0187	-0.0004	-0.0352	-0.0281	-0.0321	-0.0237
Distribution volume L	7.14	11.90	*	5.51	6.34	8.6	9.08
GH half-life min	32.8	37.1	*	19.69	24.66	21.58	27.2 (3.3)
PULSE 2							
Exponent	-0.009	-0.0357	-0.0191	-0.0293	-0.0267	-0.0254	-0.0272
Distribution volume L	*	5.76	9.37	8.49	6.16	10.10	7.31
GH half-life min	*	19.4	36.28*	23.65	25.96	27.28	26.5 (2.8)
PULSE 3							
Exponent	-0.0243	-0.0334	-0.0238	-0.0326	-0.0314	-0.0322	-0.0296
Distribution volume L	5.37	5.77	7.06	7.15	4.9	7.45	6.30
GH half-life min	28.52	20.75	29.12	21.26	22.07	21.52	23.9 (1.6)
Group mean SEM							
Exponent	-0.0227	-0.0293	-0.0215	-0.0315	-0.0287	-0.0299	0.027
Distribution volume L	6.15	7.04	8.36	7.92	5.75	8.58	7.38
GH half-life min	30.66	25.75	29.86	21.53	24.23	23.46	25.9 (2.6)

Table 2e GH half-life and distribution volume: Results following *r*-hGH 6mU/kg given as 60minute pulse infusions

The half-life was calculated from $[GH] = A_0 e^{-\lambda t}$ where A_0 is the maximum GH measured after distribution and λ is the exponent of decay. Half-life was calculated from $\ln 2/\lambda$. After excluding 2 outliers* there was no statistical difference between the three pulses and therefore results are pooled.

As first order kinetics of distribution does not describe DV during a prolonged GH infusion, steady state kinetics was used to calculate DV (infusion rate/ λ , mean plasma GH per pulse). This shows increased DV for Type 1 diabetic subjects: mean 7.38L (range 5.75-8.58); the mechanism of clearance is discussed in Chapter 7.

There was no statistical significant difference between the half-lives of the three pulses (paired student's t test: pulse 1 with pulse 2, $p < 0.7$; pulse 2 with pulse 3, $p = 0.2$). The half lives were pooled and the

Mean (SEM) half-life after 60minutes GH infusion was 25.9 ± 2.6 min

The calculated half-life after 60 minutes GH pulse was significantly prolonged compared to the overall mean value of 13.6 ± 4.6 min obtained from the bolus study (unpaired Student's t test, $p < 0.001$). Calculated mono-exponential GH disappearance curves are shown in **Figure 3.2c**.

Using Group mean t test at 60min infusion comparing data from control (Hindmarsh 1990, mean \pm SD half-life was 14.5 ± 2.9 , $n = 10$ studies) compared to 60min infusion half-life in diabetic patients, the difference (D) between the means was 1.69, Z statistic (distribution along lines) was 6.63 (13 df if > 4.21), and $p < 0.001$.

Distribution volume during pulse infusion

The DV during 60minute GH infusion is difficult to predict accurately as this is subject to changing receptor and binding protein alterations.

First order decay assumes that total GH distribution has occurred within the first 6minutes. However, DV may be altered by continuous infusion (prolonged GH exposure). DV was calculated by using steady state analysis for metabolic clearance rate (Schaefer 1996):

$$\begin{aligned} \text{MCR (L/min)} &= \lambda \cdot (\text{DV diabetic subjects}) \\ \text{and MCR} &= \frac{\text{infusion rate}}{\text{steady state plasma level}} \end{aligned}$$

For subjects HJKLMP (Tables 2.1c and appendix A2.2i)

mean r-hGH dose injected = 377 mU per hour (pulse)

= 6.28 mU per minute

therefore calculating mean exponent λ for each pulse

From section 6.0.2iv mean plasma levels during pulses

	mean GH mU/L	λ	DV L
Pulse 1	29.2	0.0237	9.08
Pulse 2	31.6	0.0272	7.31
Pulse 3	33.7	0.0296	6.30
Mean	31.5	0.027	7.38

The DV during a prolonged GH infusion increases to a mean of 7.4L. This was used for steady state calculation

3.1.0 GH clearance: 12hour (steady state) infusion

The mean \pm SEM GH level achieved during steady state (equilibrium) infusion (22.00h-08.00h) was 9.8 ± 0.4 (SD 1.5) mU/L (section 6.0.2v)

Half life: Prolonged GH exposure

Steady state was determined by ANOVA, 120minutes after the start of GH infusion (rate of 1.5mU/kg/h). The serial plasma GH concentrations reached equilibrium over the 10 hours (period 22.00h-0800h) confirming no significant drift by ANOVA. This inference was confirmed by linear regression defining a zero slope of GH measurements over the last 6 hours of the clamp (Figure 3.2d).

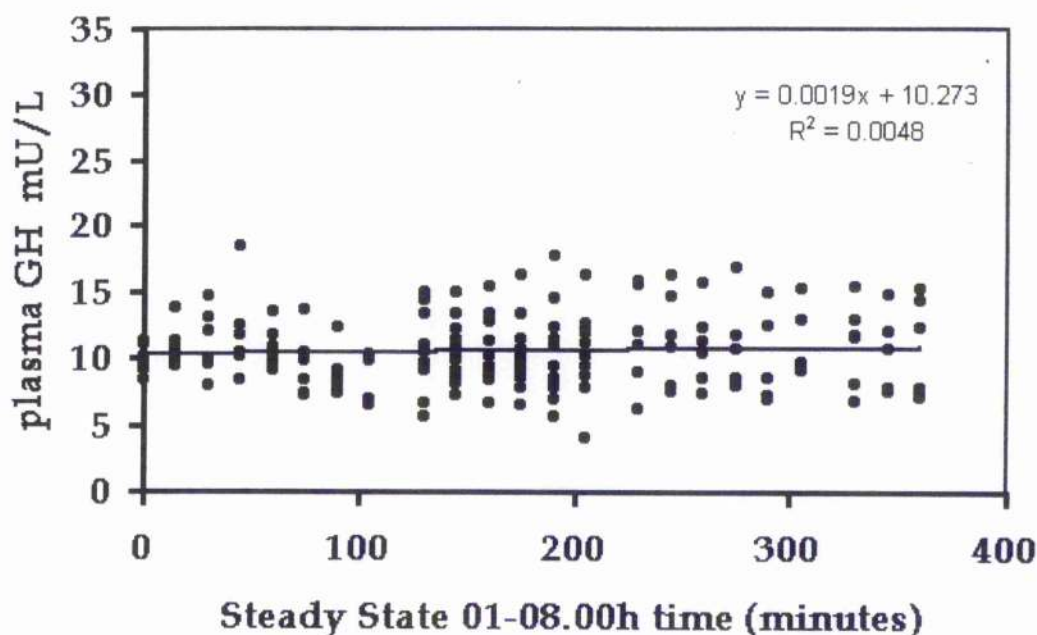


Figure 2d Plasma GH levels during steady state

Continuous 12 hour overnight GH infusion was commenced at 20.00h. This achieved steady state by 23.00h. The data shown here is for the last 6 hours after steady state achieved, which is confirmed by the linear regression defining a zero slope.

Mean plasma GH averaged 9.8 mU/L . Steady state calculations (see text) estimated decay constant and half-life as $t_{1/2}$ of 28.5 minutes. The context in which GH enters the circulation (duration of GH exposure) critically determines half-life.

Calculation for steady state

At steady state the continuous iv GH infusion rate equals the product of the measured serum GH concentration (mean over duration of infusion) and the metabolic clearance rate (MCR) (Schaefer 1996) where

$$\begin{aligned} \text{MCR (L/min)} &= k \cdot (\text{DV diabetic subjects}) \\ \text{or MCR} &= \frac{\text{infusion rate}}{\text{steady state plasma level}} \end{aligned}$$

Also at steady state the half-life (mins) $t_{1/2} = \ln 2/k$

GH infusion rate $= 1.5/60 \text{ mU/kg/minute} = 0.025$

steady state plasma [GH] $= 9.8 \text{ (SD 1.5) mU/L}$

Median weight in diabetic subjects (JLMNPQ) is 70.5 kg

Distribution volume during steady state (DV) $= 7.4 \text{ L}$

$$k = \frac{0.025 \times 70.5}{9.8 \times 7.4} = 1.763/72.3$$

$$k = 0.0243$$

$$t_{1/2} = \ln 2/k = 0.693/0.0243 = 28.5 \text{ (SD 4.3) minutes}$$

mean (SEM) GH half-life after 12h exposure is 28.5 + 1.8min

range 24.2– 32.8 minutes

These half-life data (steady state > 60min infusion > bolus) were used together to describe the relationship of GH half-life with duration of GH exposure in Type 1 diabetic subjects (Figure 3.2e) and compared to control data (Table 3.2f).

Duration of GH infusion minutes	Type 1 Diabetes GH half-life minutes (SEM)	Control GH half-life minutes (SEM)	
		Hindmarsh 1989/90	Shah 1999
0	13.6 (1.1)	8.9 (0.3)	9.8
15	-	12.6 (0.85)	-
30	-	14.0 (1.3)	-
60	25.7 (2.1)	14.5 (0.9)	-
180	-	16.3 (0.9)	-
Steady state	28.5 (1.8)	-	15.1
r-hGH dose administered	100mU bolus 6mU/kg/h pulse 1.5mU/kg/h steady state	50mU&500mU 3mU/kg/h	2-8 mU/kg bolus 1-9mU/kg/min 240 min

Table 32f GH half-life is critically dependent on duration of exposure

GH half-life data obtained in Type 1 diabetic subjects in Chapter 2 are compared with reported controls. All subjects had endogenous GH suppression before GH half-life studies. GH dose of administration varies between studies but this does not influence GH half-life.

MCR was also calculated and compared to reported normal subjects, corrected for surface area: For subjects who received r-hGH during 12hours infusion

$$\text{MCR} = \frac{1.5 \text{ (mU/kg/h)} \times 70.5 \text{ (kg)} \times 1000 \text{ mL/min.m}^2}{9.8 \text{ (mU/L)} \times 1.59 \text{ (m}^2\text{)} \times 60 \text{ (min)}}$$

MCR for Type 1 diabetic subjects = 113.1 mL/min. m²

3.1.1 GH Exposure and Asymptotic Relationship

The GH half-life in diabetic patients after different duration of exposure is prolonged at any time when compared to the non-diabetic group, for instance, half-life controls was 15.3 minutes after 60 minutes GH exposure, diabetic subjects 25.7 minutes. The time-mode of GH exposure critically extends GH half-life and this can be described by an asymptotic relationship [$y = A \cdot \ln(x) + B$] where y =half-life(mins) and x =time of exposure. A similar relationship regarding the duration of GH infusion and GH half-life has been established in normal subjects. **Figure 3.2e** shows this relationship in subjects with Type 1 diabetes and in 2 reported studies of normal data (Hindmarsh 1990 and Shah 1999).

Rebound endogenous GH

The characteristics of the rebound GH pulse after somatostatin withdrawal (t150-270mins) are detailed in Chapter 4. There was no statistical difference in rebound GH between each of the clamps studied, so data was combined to estimate the half-life of endogenous GH following rebound release in 9 clamps with a single peak.

Figure 3.2f shows the relationship between endogenous GH half-life and peak GH plasma concentrations and exposure time. A significant relationship between GH half-life and duration of exposure was demonstrated ($r = 0.8$, $p < 0.005$) whereas no significant relationship between GH half-life and peak plasma GH levels was observed ($r = 0.254$, $p = 0.5$).

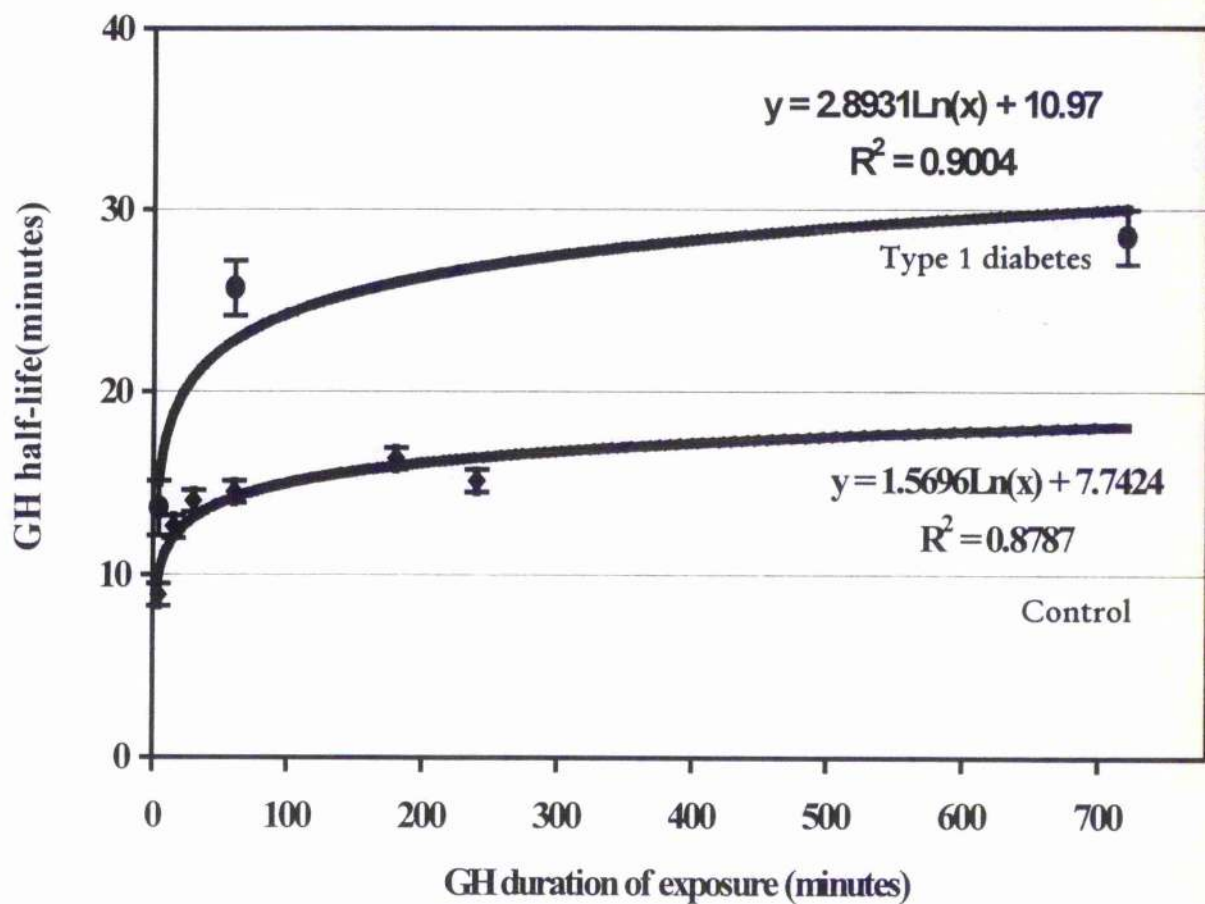


Figure 3.2e Relationship of GH exposure and half-life

Mean GH half-lives after bolus, pulse or during steady state infusions are plotted. Impact of time mode of GH entry into blood stream on GH half-life is described by the ascending hyperbolic curve $y = A \ln(x) + B$ where y = half-life and x = time of exposure. This represents the non-linear relationship between the GH half-life and the mean plasma GH concentration in both Type 1 diabetic and control subjects.

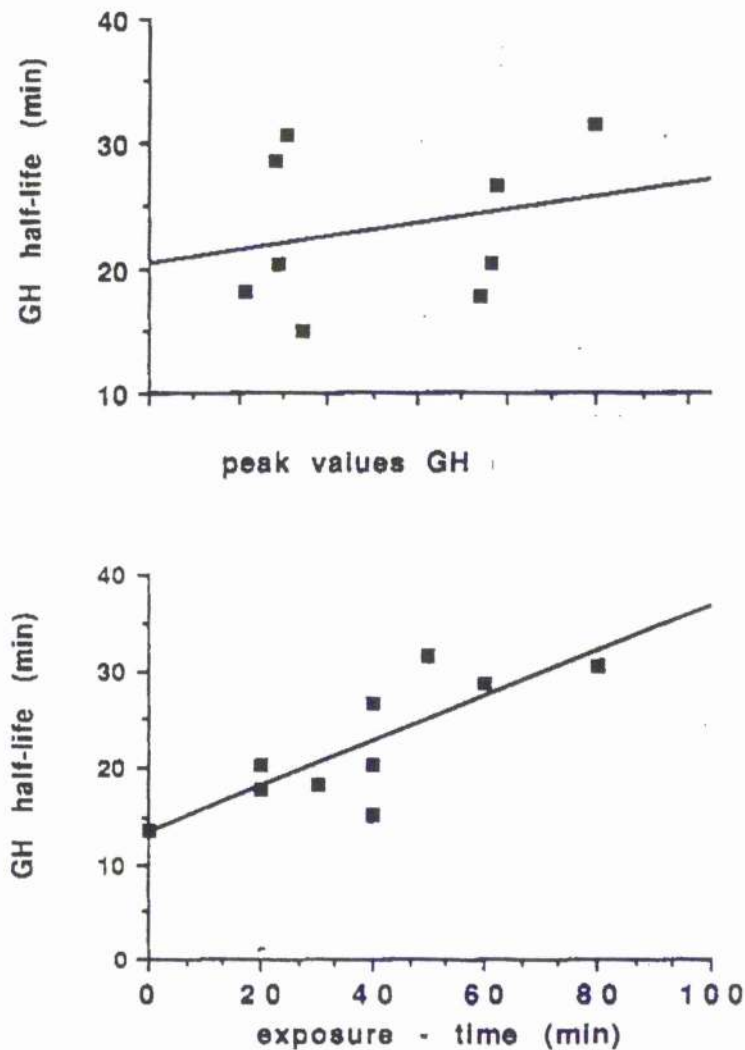


Figure 2f Increased duration of GH rebound secretion alters GH half-life, whereas peak GH amplitude has no influence

Upper panel: Endogenous GH peak levels (mU/L) following the GH rebound release after termination of SRIF infusion: No significant relationship was observed with half-life ($r = 0.254$, $p = 0.5$)

Lower panel: Duration of exposure of endogenous GH on GH half-life is strongly related ($r = 0.8$, $p < 0.005$). This illustrates again that the clearance of GH is dependent on the time mode of exposure.

3.1.2

SUMMARY and DISCUSSION

In this chapter, the disappearance parameters of GH have been assessed in young adults with Type 1 diabetes and compared to a control group of normal volunteers who underwent a similar study protocol. The parameters of GH distribution and elimination have been assessed by a) suppressing endogenous GH secretion by giving a somatostatin infusion b) administering a physiological or moderate amount of GH and c) the elimination curve of the injected GH was measured with a sensitive assay. With this approach, a mean elimination half-life ($t_{1/2}$) for *bolus* GH of 13.6 ± 1.1 (SEM) minutes (Range 11.9-19.4 min, n: 17) found in diabetic subjects was significantly prolonged compared to that observed in normal subjects (8.9 ± 0.6 , Range 6.3 -11.6 (Hindmarsh 1989) and 9.8 ± 0.8 mins (Shah 1999). However, an increase in GH half-life with increased duration of exposure is also seen in diabetic subjects (Figure 3.2c). The GH half-life calculated after iv bolus injection is significantly (50%) shorter than that assessed after 60minutes or steady state GH infusion. Thus, as the elimination properties of GH are dependent on the duration of exposure, the differences in plasma GH concentrations in diabetic subjects must reflect differences in pituitary GH secretion rates with normal controls. I have investigated this further in the next chapter.

The exact role played by the altered clearance in the GH disturbances of diabetes is unclear. The reasons are that most studies intended to evaluate GH clearance and half-life were performed several years ago with techniques such as isotopic dilution (Owens 1973) and tools such as purified recombinant human GH (r-hGH), sensitive assays and mathematical models, were not available. This ambiguous situation was compounded further as the GH clearance parameters in normal subjects was not previously settled: In fact, a wide range of GH half-lives had been reported, ranging from 7.5 to 51 minutes (Laron 1965, Kowarski 1971); using deconvolution techniques, Hartman, (1991)

Author	Normal Subjects	Dose <i>r</i> -hGH	GH Suppression method	GH t½ Mean (SEM) mins	DV L/kg	MCR
Bolus GH						
Gupta 1990	25		SRIF	11.4 to 12.6		3-4 ml/hm2
Hindmarsh 1990	10	50mU & 500mU	SRIF	8.3 (0.8)	0.054	
Garcia-Mayor 1993		400mU	SRIF	13.8 (1.6)		
Langendonk 1999	24	100mU	SRIF	4.8 to 6.6		
Shah 1999	19	2-8mU/kg	Octreotide	9.8 (0.5)	0.063	
Infusion GH						
Hindmarsh 1990	10	3mU/kg for 15mins 60mins 180mins	SRIF	12.6 (0.9) 14.5 (0.9) 16.9 (0.9)		
Lanzi 1995	7	6 hours	SRIF	18.4 (0.6)	0.055	1.7 ml/kg.min
Shah 1999	19	1-9mU/kg.min 240mins	Octreotide	15.1 (0.6)		
Endogenous GH						
Veldhuis 1991		Endogenous	Deconvolution	15.5 (0.8)		
Nieves-Rivera 1993		Endogenous	Deconvolution	19.8 (0.5)		
Shah 1999	19	Endogenous	Deconvolution	18.8 (1.3)		

Table 3.29 Published reports of GH clearance in normal subjects.

These studies estimated exogenous GH (*r*-hGH) half-life *after* endogenous GH suppression with somatostatin (SRIF) or octreotide (long acting SRIF). GH half-life measurements in normal subjects are shorter than previous reports in 1970-1980s. Endogenous GH half-life was estimated from mathematical deconvolution analysis.

estimated the mean endogenous GH half-life to be 17.1 minutes. Faria, (1989) demonstrated an endogenous GH half-life of 18 minutes after GHRH stimulated GH release when the exposure to GH was in the order of 90 minutes. Despite textbooks reporting established GH half-life of more than 20 minutes, with strict assessment of GH decay characteristics, GH half-life of less than 10 minutes has been confirmed in normal subjects (Table 3.2g).

The possibility that somatostatin could itself alter some parameters of GH distribution and clearance has previously been ruled out (Hindmarsh 1989, Faria 1989, Chapman 1991). Ongoing GH secretion during a somatostatin infusion is a possibility during GH kinetic studies, but Skamene 1984 has shown that the somatotropes are very sensitive to SRIH with adequate GH suppression even at a dose of 25 $\mu\text{g/h}$. Furthermore, I did not see not observe any other GH peaks in the individual decay curves. In addition when exogenous GH is injected, endogenous GH secretion should be even lower as GH inhibits its own secretion.

Some authors consider the half-life for GH as a non-physiological parameter. In fact the concept of half-life for a hormone or a drug is firmly rooted in our vision of the pharmacokinetics of biological compounds, so it is often difficult to realise that such a parameter has no physiological meaning because it is just a mathematical measure of the slope. Moreover, it has a mathematical meaning only in mono-exponential models and not additive over compartments (DiStefano 1982). For such reasons, mathematicians are more prone to use the concept of mean residence time (MRT) for a given hormone based on the fact that residence time moments are more useful in modelling and kinetic analysis of physiological systems (Matis 1985). However, the calculation of half-life for GH in diabetic subjects was required for deconvolution analysis to estimate GH secretory rates and therefore used in this thesis.

As a mono-exponential half-life may correspond largely to the distribution phase and therefore underestimate the irreversible metabolic clearance, bi-exponential fitting was also performed (Table 3.2d). This did not

show any significant difference from the mono-exponential half-life values derived from the analysis of GH values after the complete distribution phase following injections of exogenous GH.

Of greater interest was the duration of GH exposure that prolonged GH half-life measurements in my diabetic subjects (section 3.1.1). This shows an asymptotic relationship (Figure 3.2e) and suggests that GH half-life has a variable half-life that is prolonged and critically related with increasing GH exposure. GH half-life is increased at all exposures compared to controls (Table 3.2f): 60min GH infusion $t_{1/2}$ 25.7 ± 2.1 (SEM)mins in diabetic patients was prolonged compared to the bolus data (unpaired Student's *t* test, $p < 0.001$) and to the 60-minute exposure values obtained in controls (14.5 ± 2.7 (SD) minutes, group mean *t*-test $p < 0.001$).

My data for 12hour GH infusion half-life was utilised from subjects attending the GH signal study. Even with this prolonged infusion, a **maximum GH half-life of 28.5 ± 1.8 (SEM) minutes** was confirmed. The duration of detectable serum GH concentrations usually lasts between 40-100 minutes in normal individuals (Hindmarsh 1990) and these authors consider that infusion studies to determine GH half-life do not represent the usual *in vivo* situation, particularly as elevated serum GH concentrations for 3 hours or more are non-physiological.

Previous studies on exogenous GH half-life disappearance rates from the circulation in diabetic patients compared to controls (normal subjects) are controversial: Owens (1973) reported a mean half-life of 21.8 minutes not significantly different from the 19.0 minutes found in normal subjects after the 'priming dose-constant infusion' technique of unlabelled hGH on increasing subsequent GH infusion rates covering the normal range of 10-100 mU/l ($5-50 \mu\text{g/L}$); in contrast, Boucher found significantly increased half-lives of ^{131}I -labelled hGH prepared by the Raben technique given as a bolus in diabetic patients compared to the half-life in non-diabetic subjects (Boucher 1969). Some of these discrepancies may be explained by the use of different assay

systems to measure GH (Celniker 1989) and by the method of half-life determination of unlabelled or radio-labelled GH using different analytical models. In addition, the variable endogenous GH secretion during studies where endogenous GH was not suppressed could account for the variability of earlier published reports. A more direct comparison of my data can be obtained from the work of Hindmarsh (1989,1990) where endogenous GH was suppressed with somatostatin. Other authors reporting GH half-life data after somatostatin suppression also confirm the shorter half-life in normal control subjects (bolus $t_{1/2}$ 4.8-6.6 mins Langendonk 1999).

More recently Shah (1999) has carried out a series of studies including bolus and 240minutes GH infusion in normal adult volunteers after endogenous GH suppression with Octreotide (long acting SRH). They also found a varying GH half-life that was predominantly related to the time mode of hormone entry into the circulation rather than gender or oestradiol concentrations. Their findings confirm a changing GH half-life with duration of infusion (240mins equilibrium infusion mean (SEM) $t_{1/2}$ 15 ± 1 mins > after bolus injection was 9.8 ± 0.8 mins). Other authors using a more prolonged GH infusion have found a maximum half-life of under 20 minutes (6hours GH infusion $t_{1/2}$ 18.4: Lanzi 1995). These normal data provide further evidence that compared to normal subjects, GH half-life in my diabetic subjects is prolonged at all exposures (and Figure 3.2e).

Some authors measured the metabolic clearance rate (MCR) of GH in patients with Type 1 diabetes compared to a normal control group: Lipman (1972) found lower MCR in young, newly diagnosed patients with Type 1 diabetes after a bolus injection of ^{131}I -labelled hGH as a priming dose followed by ^{131}I -labelled hGH infusion at a constant rate for 90-120 minutes; Sperling (1973) described significantly reduced MCR of ^{131}I -labelled hGH after constant infusion maintained for 150 minutes in patients with Type 1 diabetes. In contrast, Navalesi (1975) reported a normal MCR in diabetic patients after a bolus administration of ^{125}I -labelled hGH. Again the contribution of

endogenous GH secretion while performing clearance studies of GH has been pointed out (Owens 1973, MacGillivray 1970) and could explain the wide variation in half-lives reported.

To examine the model for the GH disappearance profiles, Garcia-Mayor, (1993) fitted real data of GH clearance from normal and uraemic patients to a multi-exponential model following Marquart's iterative algorithm. The model was estimated by non-linear, weighted, least squares regression and checked using the criteria (1) visual inspection of graph plots and an analysis of variance between the original data and estimated model (2) the squared correlation coefficient (r^2) was always higher than 0.98. With such a procedure, the best-fit model was bi-exponential, with a first phase of distribution and a second phase of elimination.

My data was analysed using both mono- and bi-exponential models after log-transformation of GH data and the results were not significantly different using either model (Table 3.2c,d). Although, ultrasensitive GH assays have recently become available and was not used here, a more sensitive assay would not introduce imprecision in the calculation of GH half-life, but may be very useful for the mathematical validation of models employed. The mono-component GH half-life value estimated after a single bolus is not proportionate to the peak serum GH concentration. After a single bolus iv injection into a somatostatin-suppressed (GH deficient) circulatory pool, an accelerated half-life value of initial GH disappearance would be due to rapid diffusion and advection of GH molecules (Keenan 1998).

In contrast, the 60minute pulse and continuous GH infusion represent more closely the context of GH entry into and removal from the bloodstream. The observations in this chapter suggest that GH half-life in diabetic subjects did not increase significantly after an exposure of 60minutes in diabetic patients. The available clinical literature remains unclear about the presumptive regulation of GH half-life and volume of distribution in Type 1 diabetes and there may be several explanations for this observation. Although the GH peak

levels were higher for GH infusion (60min) than they were in the bolus study, it is unlikely that the GH levels in the physiological range would show a significant effect on hGH half-life since in other studies the half-life was not altered by the GH amplitude (Faria 1989, Hindmarsh 1990, Shah 1999). Furthermore, my data shows that there is no relationship between the plasma concentration and the GH half-life, which confirms data described by Owens (1973) that the metabolic clearance rate of GH is independent of plasma concentration. Some authors previously showed that the kinetics of GH hormone disposal had a strong positive correlation with body mass index (relative obesity) in humans (Schaefer 1996) and various clinical disorders, particularly renal or hepatic disease (Haffner 1994, Cuneo 1995). My subjects had normal renal and hepatic function and were not obese (Table 2.1a,c and 3.2a).

Decreased renal GH clearance has also been suggested as a possible mechanism for high plasma GH concentrations in diabetes mellitus but this has been rejected by a study showing that the renal clearance of GH in children with diabetes mellitus is increased compared to normal children (Hourd 1991). This is not likely to be the explanation for the prolongation of the GH half-life. In addition, a study looking at uraemic patients, showed that GH clearance is not impressively reduced considering the severity of kidney damage in such subjects (Garcia-Mayor 1993). This suggests that either the kidney plays a minor role in GH clearance compared with the liver and peripheral tissues or that most of the kidney clearance for GH is preserved even when this organ is no longer able to accomplish the minimal detoxifying functions compatible with life.

Studies of the size variants GH in diabetes mellitus have revealed a higher proportion of monomeric GH than in normal men (MacFarlane 1986) and Baumann reported that different molecular weight GH variants as well as GH monomers and dimers may have different clearance rates (Baumann 1985,1986). However, in this work I used recombinant hGH (r-hGH, 22kDa),

so variants cannot be the explanation here. In addition there was no difference in the half-life of GH measured by three different assays, one of them the Hybritech IRMA, which specifically detects monomeric 22-KDa GH (Bowsher 1990, **Appendix 3e**).

The biological action of GH is thought to be initiated by the binding of the hormone to specific receptors in the cell membrane and this appears to represent the major way in which hormone is cleared from the circulation. Studies using cultured rat adipocytes have demonstrated two pathways for intracellular processing of GH which are independently regulated - the non-degradative pathway: GH is taken up into the cell and then simply released intact back into the extra-cellular space. This process takes about 30 minutes to complete (Gorin 1984, Roupas & Herington 1987 a,b). Secondly, there is a degradative pathway - GH is taken up into the cell then broken down. This process is much slower and is highly susceptible to prior exposure of the cell to GH. Both processes occurring together would support the observations of the variable GH half-life seen with duration of exposure (asymptotic relationship) in both diabetic and control subjects (**Figure 3.2e**). A similar model has been proposed for the internalisation and processing of the insulin receptor (Marshall & Olefsky 1983).

As assays for GH-BP are now available, discussions about the causes of prolonged half-life in diabetes now focus on protein binding of GH (GH-BP) and the receptor changes (Amit 2000). The presence of a physiological, finite capacity, but high affinity, GH-binding protein in human plasma would logically account for the variable half-life estimates (Veldhuis 1993). Long exposure to GH may more extensively saturate the GH binding protein (GH-BP) which is known to increase the half-life since protein-bound GH is cleared significantly slower than free hormone (Baumann 1994). It has been shown that in experimental animals each burst of GH pulse is followed by a wave of receptor turnover and an increase in serum binding protein (GH-BP) which occurs 60 minutes after the GH peak (Amit 2000).

Hochberg (1994) described the 24 hour profile of plasma GH-BP and its correlation to GH pulsatility in normally growing children in early puberty and showed that plasma GH-BP levels fluctuated rapidly (GH-BP pulses were seen within 30 min) in relation to the pulsatility of plasma GH levels. It has also been shown that the plasma concentration of circulating high affinity binding protein (reflecting the relative abundance of the extracellular domain of the hepatic GH receptor) is low in insulin-dependent diabetes, with the most striking decrease being during puberty (Mercado 1992). This would suggest that reduced receptor binding and internalisation diminish GH receptors in Type 1 diabetes resulting in delayed clearance.

My results also show that short-term alterations in glycaemia and insulinaemia do not alter GH clearance parameters of half-life and distribution volume (section 3.0.8, Table 3.2c). There are theoretical reasons why the GH disappearance rate from the circulation may be altered by the prevailing glycaemic and insulinaemic state. Hyperglycaemia promotes glycation of circulating proteins such as haemoglobin and plasma proteins and a study has reported that the GH half-life of disappearance from the circulation was delayed after periods of hyperglycaemia (Mullis 1988). Although the decline of unglycated and glycated albumin is identical, fructosamine (ketamine linked glucose to all plasma proteins) measured by the fructosamine assay shows a delayed disappearance after periods of hyperglycaemia (Mullis 1988) therefore non-enzymatic glycation of the GH and/or other forms of the half-life important proteins may result in an increase of GH half-life.

Insulin deficiency could result in a reduction in GH receptors and hence clearance by the receptor and it has been shown that in the diabetic rat, binding of GH in the liver is reduced and can be restored by insulin (Baxter 1980b). Conversely, as hyperinsulinaemia stimulates the GH-receptor expression (Baxter 1980b), one could expect a possible decrease in the calculated GH half-life compared to normoinsulinaemic conditions. However, short-term metabolic changes (hyperglycaemia and/or hyperinsulinaemia) did not result in

significantly different GH half-life measurements. Thus, in diabetic patients, the previous concerns of fluctuating insulin levels resulting in a alteration of GH receptors and hence clearance by the receptor; size variants of GH and clearance rates of different molecular weight GH variants and GH monomers and dimers have not been shown to affect the GH clearance in my studies.

This chapter shows that the mean volume of distribution was also increased in the diabetic patients (6.1 L, range 4.7-7.8) compared to the values reported in the controls (3.5 L, range 2.02-5.6, group mean test $p < 0.0005$) (Hindmarsh 1989). This represents a volume of distribution of GH in diabetic patients of 8.9% body weight (5.3% in normal subjects). Other hormone kinetic studies in Type 1 diabetes report increased volumes of distribution for adrenaline (Dejgaard 1989) but unaltered volumes of distributions for GH (Navalesi 1975).

The long-standing controversy whether clearance is correlated with body surface area (BSA) or with body weight has so far remained unresolved. The choice between use of BSA or body weight for calculating distribution volume of GH is dependent on assumptions about the nature of GH distribution. Plasma volume appears to correlate well with body weight, while the extracellular volume (ECV) correlates with BSA (Boer 1984). Refetoff (1970) claimed that purified pituitary GH is distributed partially in the ECV. As this is contradicted by other studies, using recombinant human GH, in which the distribution space was found comparable to the intravascular space (Albertsson-Wikland 1989), I have used the parameter of body weight. The larger distribution volume in the earlier study (Refetoff 1970) may also have been influenced by the detection of GH peptide fragments in RIA, which are not measured in 2-site IRMAs. Although previously body fat and sex hormones were thought to influence GH distribution space, recent studies confirm that neither affects clearance (Langendonk 1999, Shah 1999).

The mechanism for altered GH distribution volume found in diabetic patients are several. The increased permeability of the basement membranes in

diabetic subjects is well described and may precede structural changes (Parving 1975). In addition, elevated levels of endothelin in patients with diabetes as a result of endothelium cell damage has been reported (Takahashi 1990). The endothelium cell damage is likely to be an additive factor in increasing the permeability of the vessels.

These data on half-life and volume of distribution of GH do not provide any information concerning distribution of GH in different compartments after bolus or prolonged exposure, nor do they tell us anything about renal or metabolic clearance or changes in the compartmentalisation of GH during the 'emptying' phase. The use of massive bolus of GH or a constant infusion could induce a disturbance in the normal distribution and degradation/clearance of GH. In fact, supraphysiological doses of GH might alter the distribution of GH between compartments and/or saturate the internalisation/degradative process of tissue GH receptors (Roupas & Herington 1987a), then redirecting GH toward the extracellular space. Although the observation of changes in the disappearance rate with prolonged exposure requires further investigations, the data presented are very important for the purpose of deconvolution analysis of plasma GH profiles to determine GH secretory rates. I have shown that the prolonged GH half-life measured in diabetic subjects is a direct function of the duration of GH exposure (Figure 3.2e). This suggests that GH pulse secretion in diabetic subjects must also be prolonged: either there is a persistent pulse amplitude, increased pulsatility or there is a constantly raised basal GH secretion.

To elucidate whether indeed abnormal GH pulse secretion exists in diabetic compared to normal adolescents, I have analysed a series of plasma GH profiles in the next chapter.

Summary

In Type 1 diabetes, GH half-life is increased when compared to control subjects and varies with duration of GH exposure; short-term alterations in glycaemia and insulinaemia do not alter GH clearance parameters of half-life or distribution volume

CHAPTER 4

ANALYSIS OF PLASMA GH LEVELS DISTRIBUTION AND DECONVOLUTION

4.0.0

INTRODUCTION

Several hormones are released into the blood in a pulsatile fashion, that is, in short bursts that cause a rapid increase in the circulating concentration. The concentration then declines due to excretion, chemical breakdown, and redistribution into other compartments. Based on a series of observations of the level of the hormone in blood, investigators desire to quantify characteristics of these pulses, which are timing, amplitude, and rate of decay.

Detecting and describing peaks in a series of hormonal data is complex. As GH fluctuates episodically (**Figure 4.3a**), it is difficult to ensure an adequate measure of the mean level, enumeration of pulses and peak detection. The literature appears to contain a bewildering large array of methods for pulse analysis. However, many of these turn out to be variants of the original Santen and Bardin's technique (1973). Santen and Bardin showed that a series of samples was a better estimate of the mean. The number of samples required depends on the accuracy needed, physiological variability of the levels and the errors of the measurement process. Most methods of the analysis of growth hormone profiles require the ability to identify and characterise the pulses of the hormone in a mathematically explicit and statistically defined manner (Veldhuis 1988a). Techniques such as pulse counting or peak picking or modelling, using a range of computer programmes are many, each having their limitations.

In my analysis of GH profiles obtained from children with Type 1 diabetes and controls (section 2.0.7-2.1.1) I have used a distribution method, deconvolution analysis and time series analysis.

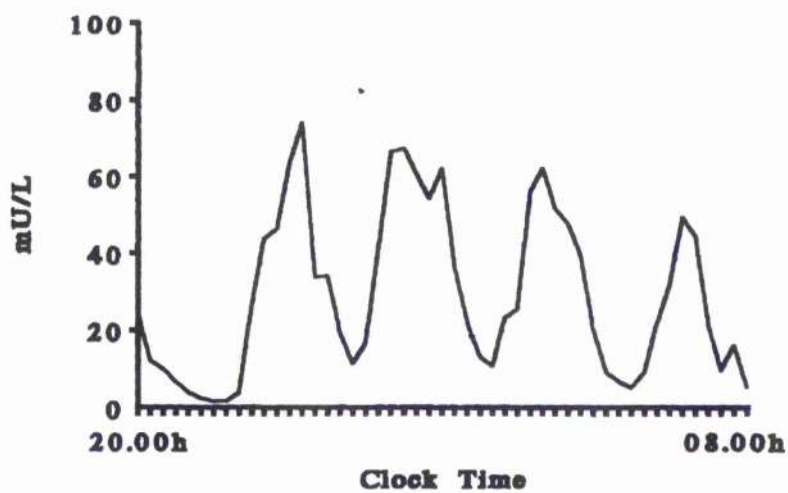
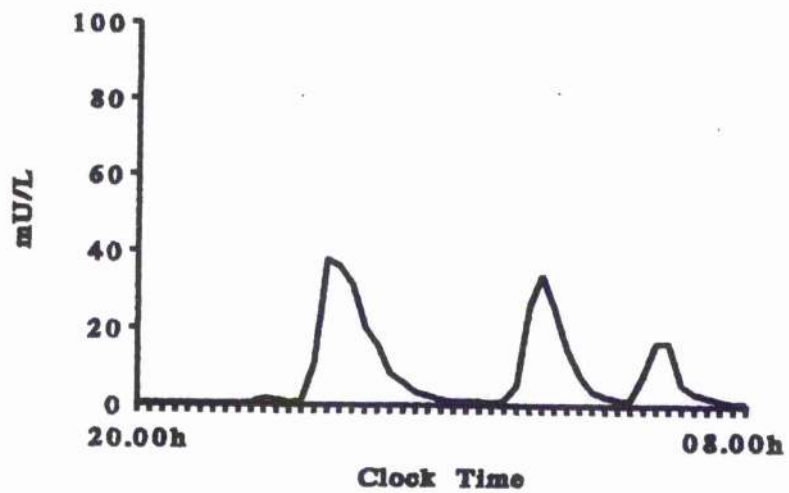


Figure 3a Spontaneous plasma GH levels.

Examples of GH profiles in control (normal healthy) (upper panel) and diabetic adolescent (lower profile).

4.0.1 Method of distribution analysis

A novel and unbiased application to estimate baseline, mean and peak GH levels based on probits (probability units) has been described by Matthews, 1991. This method does not depend on other factors such as assay sensitivity, or the number of observations and allows comparison between subject groups with particular characteristics.

Matthews (1991) has described using a method of cumulative distribution and probability to provide an objective assessment of GH profiles. The log-transformed GH concentrations (regardless of their temporal attributes) are sorted and allocated to class intervals. The number of observations in each interval are then determined and expressed as a percentage of the total number of samples drawn in the study period. The cumulative distributions are transformed into discrete probabilities (linear probits), which are then linear and amenable to regression analysis.

The methodology has several advantages

1. the method is unbiased
2. all the data collected in a GH concentration profile can be used
3. a straightforward visual description of the data can be generated
4. the data can be pooled in a similar manner to that devised for autocorrelation and Fourier transformation, hence descriptions of populations can be made
5. the technique uses standard statistical methods for the construction of the frequency distribution and for assessing the significance of differences between frequency distributions obtained from different groups of patients and requires no special computer programming

Probability analysis gives an estimate of the trough, which can be defined in terms of percentage occupancy - the "Observed Concentration" (OC5) which is the threshold at or below which the hormone concentrations are estimated to be 5% of the time; the mean which can be defined as percentage occupancy at

50% of the time (OC50); and peak can be defined as being a percentage occupancy above 75% (OC75=1SD) or 95% of the time (OC95=2SD).

The standard deviation of the observations can be calculated from the slope of the probit (since 5% to 95% encompasses ± 2 standard deviations). Analysis of data from groups of individuals with a particular condition can be made and the difference between the lines can be assessed using 90% confidence intervals of the regression lines (Scheffler 1962), where it can be demonstrated that if the intervals from comparative lines do not overlap they are different with a null hypothesis probability of $p < 0.025$.

4.0.2 Deconvolution analysis

At any given time, plasma GH concentration is a result of GH secretion and elimination. Efforts to separate the effects of secretion and metabolic clearance have led to the development of deconvolution techniques (Veldhuis 1987). In addition the use of deconvolution analysis has made it possible to estimate the frequency, amplitude, mass and duration of GH-secretory episodes which give rise to GH concentrations in circulating blood in a variety of physiological and pathological states. The time course of the effect of physiological variables on GH secretion may be determined more precisely by calculating GH secretion rates.

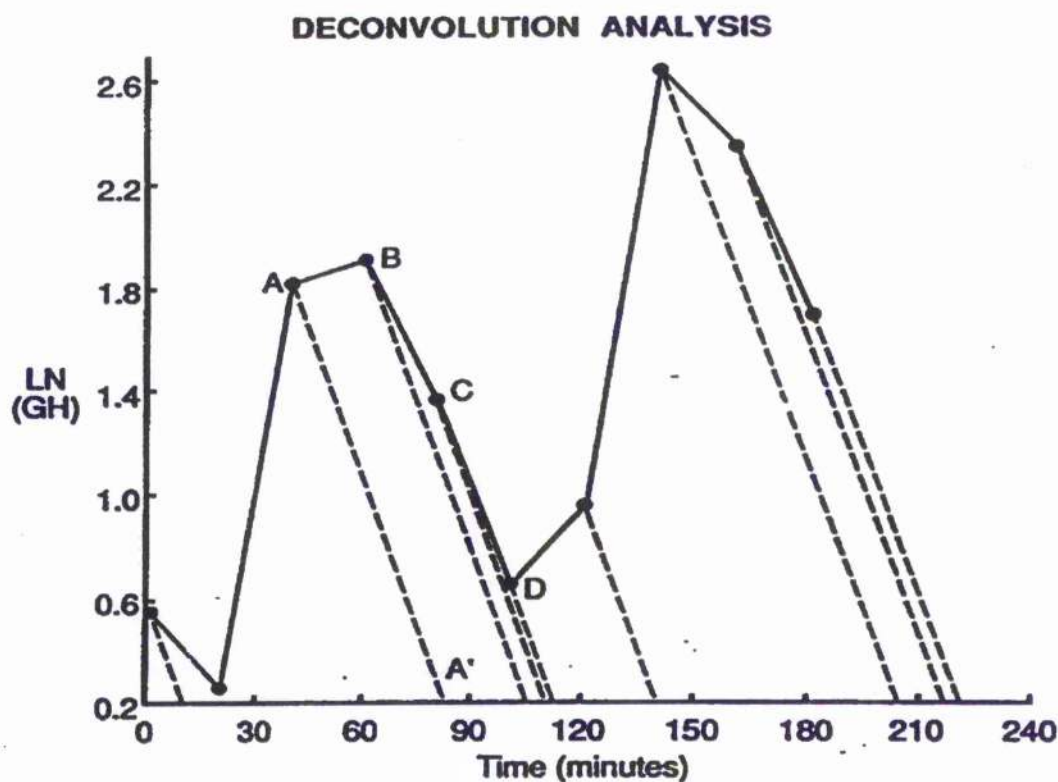
Two general categories of deconvolution methods have been devised - either methods using a previously calculated hormone half-life or methods to calculate both hormone half-life and secretion rates simultaneously assuming a specific shape of the underlying secretory event. Thompson, 1972 proposed a method to estimate secretion on the basis of integrated serum GH concentration (ICGH) and the metabolic clearance rate. Finkelstein (1972) calculated the secretion per peak on the basis of serum GH levels, the GH half-life ($t_{1/2}$) and distribution volume, according to the method described by Hellman (1970) for cortisol secretion. Thereafter, various deconvolution techniques were developed in which secretion and elimination were separated as two

components of a GH profile over time (Veldhuis & Johnson 1988a, Oerter 1986, Veldhuis 1990 and 1992).

Studies comparing previous methods of deconvolution found significantly different results in the calculation of GH secretory rates and the differences were large as a factor of 3.5 (Kuilboer 1992). This suggests that significantly different assumptions were used by the four methods previously described (THOM, HEL, DECONV, PULSE detailed in Appendix 1b).

These differences were mainly due to the calculation of GH clearance which were 2.2 times higher in THOM (from MCR used in THOM, a $t_{1/2}$ of 11.8min assuming distribution volume of 7%; PULSE uses biexponential $t_{1/2}$ expressed as monoexponential of 18.9min (Faria 1989)); area under curve (THOM) or area under baseline (HEL) which differed by 1.6 times; the use of all data points by THOM whereas HEL uses part of peaks detectable by PULSAR. The assumption inherent to Hellman's equation is that secretion takes place only during a rising serum concentration level. In contrast it has been shown by Veldhuis (1987) that secretion can also take place during the downslope of a serum GH peak. Veldhuis has used a multiple parameter deconvolution method to estimate both GH secretion and clearance rates simultaneously and hypothesised an inferential model of GH secretion (interaction of multiple pulses of hypothalamic GHRH stimulating the pituitary gland during a period of diminished somatostatin secretion) (Hartman 1991). Van Cauter (1992) also observed a multiphasic pattern of GH secretion using a different deconvolution algorithm. All differences contribute to the imperfect correlation between these methods. Besides the differences among studies, there are also up to 4-fold differences in GH MCR and/or $t_{1/2}$ using a single method of estimation applied to different subjects (Thompson 1972). Difference in MCR turn out to be important for causing differences in estimated secretion. There is little agreement among the various studies.

In most of these studies endogenous GH secretion was not suppressed, which would account for some of the variations observed. In addition, the use



Courtesy of Matthews 1989

Figure 4a (part 1) Principles of Deconvolution

A segment from a 24 hour GH profile showing two GH pulses. The serum GH concentration measured at point A would subsequently decline to AA' if no further GH was released from the pituitary gland and this point could be estimated from the known half-life of GH. The plot is linear because of the Ln transformation. At point B the serum GH concentration is higher than A which must mean that further GH has entered the circulation between points A and B. The amount secreted is proportional to the distance between the "stripping curves" (the dotted lines). This process is then repeated with the next serum GH concentration and repeated throughout the data array. At point C the dashed lines come closer together and eventually overlap at D. This indicates that secretion of GH ceased at point C and that the subsequent concentration at D represents the clearance of the hormone from the circulation since the value can be entirely explained by the half-life of GH.
(taken from reference)

KEY Solid line: actual GH profile. Dashed lines: "stripping curves"

of supraphysiological GH doses over a long period of time in the infusion studies has been shown to alter the clearance rate leading to a longer half-life (Hindmarsh 1989, 1990). There is no consensus between investigators concerning differences between sex and age groups. This suggests considerable biological variation among healthy subjects. During the last decade investigators have examined GH decay after endogenous GH suppression and found a shorter GH half-life in normal subjects than previously reported (Table 3.2g).

4.0.3 Deconvolution model by Hindmarsh

The deconvolution formula was derived from the methodology of Turner, 1971. The delivery rate is an iterative deconvolution whereby the estimate of the declination of GH (based on fixed or variable half-life) is subtracted from the residual measured concentration. The total secretion observed is the integral of secretion rate and time. The technique requires prior knowledge of the mode of secretion of a hormone (Veldhuis 1988b) or alternatively a measure of the half-life and distribution space of that hormone within the circulation (Hindmarsh 1990). The principle of the deconvolution model is illustrated in Figure 4.4a, which represents part of a 24-hour profile. Two GH pulses are depicted. Serum GH concentrations are Ln transformed. The performance of this deconvolution model has been verified and reported by Hindmarsh, 1990. This deconvolution model attempts to define as accurately as possible the circumstances that affect GH clearance characteristics and it has been used in this Thesis.

Chapter 3 has confirmed that the GH half-life in adolescents with Type 1 diabetes is prolonged compared with normal subjects studied under similar conditions. In order to determine whether alterations in GH secretion also exists, the calculation of GH half-life and distribution volume were applied in the Deconvolution analysis of overnight GH profiles obtained from adolescents with Type 1 diabetes and puberty-matched control. The changes in GH secretion during euglycaemia and anticholinergic suppression were

also examined in diabetic subjects. The subjects and study design are described fully in Chapter 2 (GH secretion study, section 2.0.7).

4.0.4 Time series analysis

Spectral analysis

Spectral analysis is a standard well understood statistical technique for which adequate computer software exists. Murdoch (1985) used "spectral analysis" to break down a hormone series into a set of frequency (or equivalently periodic) components each with their characteristic amplitude. These components comprise the periodogram. Dominant and harmonic frequencies may be derived, and tests of statistical significance are available. Spectral analysis does not take into account the known magnitude of the noise component of the assay. It is hard to derive satisfactory summary statistics such as mean amplitude or interpeak interval since the periodogram is a set of values comparable in number to the original series.

Three minute moving average

Individual data sets are averaged using a 3-min moving average; this reduces the size of any fluctuations shorter than 3 min (noise) at the expense of reducing the amplitude of any longer oscillations present.

Fourier Analysis

Time-series data consists of signals together with experimental and assay errors or "noise." The ability to detect the signal depends on the signal/noise (S/N) ratio (Chatfield 1975). Techniques can be used to maximise the S/N ratio and describe the signal.

Fourier Transform (FT) analysis is an unbiased method of examining all the oscillatory signal within an array. Any complex waveform can be analysed as the sum of a series of sinusoids $F(h)$ calculated from the data:

$$A(h) \sum_{i=0}^n \cos 2 \pi h(x_i/x_n) f(x_i)$$

$$B(h) = \sin 2 h (x_i/x_n) f(x_i)$$

$$\text{and } [F(h)] = A(h) + B(h)$$

The components have amplitude and frequency attributes. Fourier Transform analysis allows the power of each oscillatory function to be calculated and displayed as a histogram of power versus frequency. This is termed a power spectrum. Fourier transforms can thus produce composite spectra of data showing all the dominant and sub-dominant harmonics together with an estimate of power and amplitude. The data can be pooled using parametric statistics to yield spectra that are composite for the group of data. Fourier analysis has been used in this chapter to analyse GH secretory data for assessment of pulse periodicity and amplitude.

4.0.5 Statistics

The results are expressed as mean \pm SEM, unless otherwise stated. Statistical analysis was carried out using Student's paired and unpaired t-tests. Mann-Witney U test was used to compare between normal and diabetic subjects by sex and at each puberty group (Appendix 4c).

4.0.6

RESULTS

Eighty-five overnight plasma GH profiles were analysed: 34 from control subjects; 15 Normal Regime (NR), 29 euglycaemic clamp (EC) and 7 pirenzepine (PIR) profile nights from diabetic subjects.

4.0.7 Plasma GH characteristics

Mean GH concentration, trough and peak estimates from Distribution analysis were calculated for NR, EC and PIR. Plasma GH concentrations were log₁₀-transformed for analysis. The log-transformed GH concentrations (regardless of their temporal attributes) were sorted into ascending order and allocated to class intervals. The same class intervals were used for the plasma GH concentrations for both diabetic and control groups (Table 4.3a). The class interval chosen reflect the range of measured hormone but quantiles are not critical. As the sensitivity of the GH assay used was 0.3mU/L and this accuracy is limited by the assay CV of 8-10%, the lowest class is commenced at 0.5mU/L and the range reflected the peak GH (60-100mU/L). As pooled data from several individuals was used, the same set of class intervals was used throughout. The number of observations in each interval was then determined and expressed as a percentage of the total number of samples drawn in the study period: 49 samples at 15minute intervals were taken over a 12 hour overnight period. This percentage is displayed as a frequency distribution or domain profile and then as a cumulative distribution plotted as sigmoid ogives.

The cumulative distributions can also be transformed into discrete probabilities (linear probits), which are then linear and amenable to regression analysis. The significance of any difference between the frequency distribution of various groups can be assessed by ordinary parametric statistical methods.

Class Interval	Plasma GH Concentration mU/L	Log ₁₀ [GH]
1	0.5	-0.3
2	1.0	0
3	2.0	0.3
4	5.0	0.7
5	7.9	0.9
6	10.0	1.0
7	15.8	1.2
8	20.0	1.3
9	30.0	1.5
10	40.0	1.6
11	50.0	1.7
12	60.0	1.8
13	100.0	2.0

Table 3a Class intervals used for distribution analysis of plasma GH reflects the range of levels encountered in overnight adolescent GH profiles.

These intervals are not critical but the same intervals were used for diabetic and control subjects.

Plasma GH concentrations were log-transformed for analysis as GH concentrations are geometrically distributed.

Plasma GH mU/L	Type 1 Diabetes Distribution frequency %			Control Distribution frequency %
	NR	EC	PIR	
0.5	0	0	0	14
1.0	0	8.2	12.2	16
2.0	14.3	16.3	22.4	16
5.0	14.3	14.3	28.6	10
8.0	8.2	2	16.3	8.2
10.0	4.1	14.3	2	8.2
15.0	14.3	10.2	8.2	8.2
20.0	12.2	10.2	4	8.2
30.0	12.2	8.2	4	3
40.0	8.2	6.1	2	3
50.0	8.2	6.1	0	2
60.0	4.1	4.0	0	3
100.0	0	0	0	0

Table 3b Distribution Frequency Profile of overnight (20-08.00h) plasma GH

All the observations of concentration (regardless of their temporal attribute) were sorted into ascending order. The number of observations in each interval were then determined ("dwell times" or observed concentrations) and expressed as a percentage of the total number of samples drawn in the study period (each subject = 49 samples overnight).

The data from all puberty groups was pooled: Number of subjects: Controls=39, Diabetic NR=15, EC=29, PIR=7. The same set of class intervals was used throughout and the data displayed as a frequency distribution.

Key: NR=normal insulin regime EC= overnight euglycaemic clamp
PIR= overnight EC after pirenzepine was administered

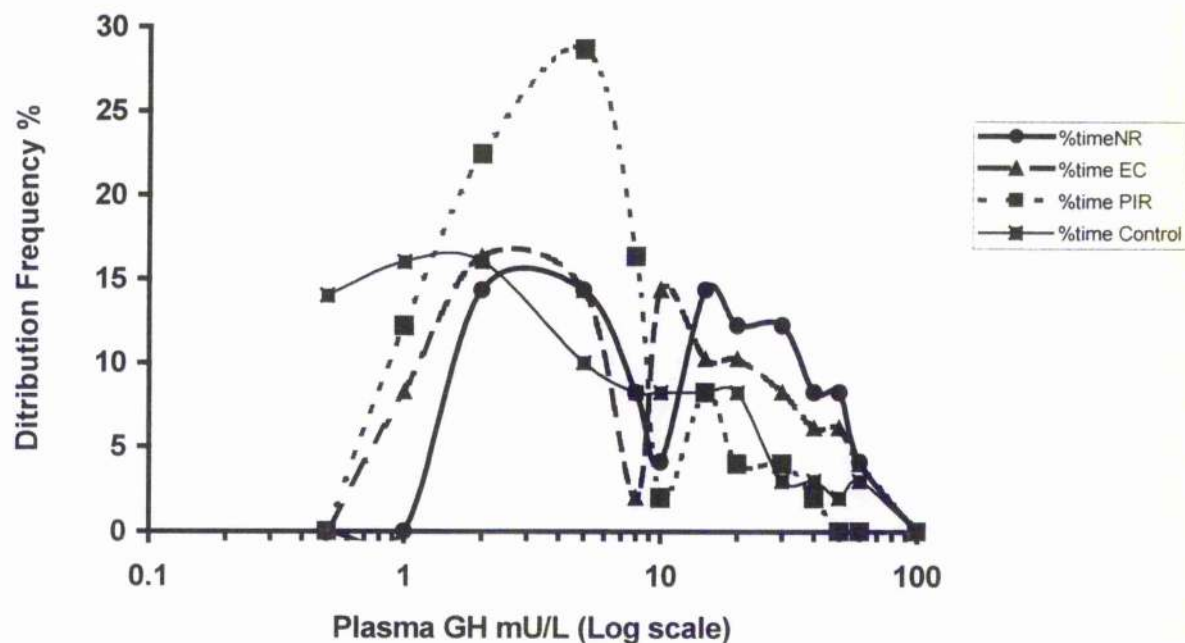


Figure 3b "Domain profiles" of Distribution analysis

Distribution analysis allows comparison of data between groups and is illustrated as percentage occupancy time (observed concentration "OC") in each dwell domain.

The occupancy in normal subjects and pirenzepine treated group is greatest at low GH concentrations. In contrast, diabetic profiles show occupancy shifted toward higher GH concentrations. .

Distribution profile

The dwell domains after distribution analysis for each group of subjects are shown in Table 4.3b and displayed as "domain profile" in Figure 4.3b.

These data illustrate that GH concentrations remain low for control subjects for a significant part of the overnight profile, compared to diabetic subjects.

Cumulative frequency profile

The distribution frequency is converted to cumulative frequency (Table 4.3c) and this can be displayed as a cumulative "frequency profile" which is sigmoid. The data from diabetic subject (NR) and control subject (Figure 4.3c) and diabetic EC and pirenzepine are shown plotted as cumulative frequency distributions (Figure 4.3d).

These data form ogives that demonstrate graphically the differences in concentration frequency overnight. Estimation of the OC parameters can be made from such plots.

Probit analysis

The cumulative frequency data can be readily analysed by probits or a probability distribution. These data can be plotted on to 'probability paper' (Chartwell paper Ref. 5571, HW Peel & Co Ltd, Greenford, UK) or by use the probit transform tables (Fisher and Yates, 1967) and calculations. The data can be used between 1-99% cumulative probability. Table 4.3c illustrates the process. The OC parameters can be estimated easily from the cumulative distribution. The probit transformation is shown on Tables 4.3c. The original probit plots are shown in Figure 4.3d. To estimate trough occupancy (OC5), as the minimum dwell time is linear, the [GH] at 5% was estimated by linear regression. The performance model is illustrated by plasma GH concentrations from the overnight GH profiles from diabetic (NR) and control subjects in Figure 4.3e.

Plasma GH	Type 1 Diabetes Cumulative Frequency %			Control Cumulative Frequency %
	NR	EC	PI	
0.5	0	0	0	14
1.0	0	8.2	12.2	30
2.0	14.3	24.5	34.6	46
5.0	28.6	38.8	63.2	56
8.0	36.8	40.8	79.5	64.2
10.0	40.9	55.1	81.5	72.4
15.0	55.2	65.3	89.7	80.2
20.0	67.4	75.5	93.7	88.4
30.0	79.6	83.7	97.7	91.4
40.0	87.8	89.8	99.7	94.4
50.0	96	95.9		96.4
60.0	99.9	99.9		99.4

Table 3c Cumulative distributions compare “dwell times” between pooled data.

Continuous summation (cumulative percentage) is amenable to transformation into linear probits and this is amenable to regression analysis.

Transformation calculations are given in the tables below.

Key for transformation tables

CF	= Cumulative frequency
EP	= Empirical probits (derived from statistical tables, Fisher & Yates 1953)
PP	= Provisional Probit This is obtained from the regression line on probability paper. This calculates mean value and A co-ordinates A (when $x=0$, y = the minimum PP) Maximum PP is at maximum GH concentration The points of intersection at x provide the provisional probits Values for the GH concentrations are then read off
N	= Provisional Probit x cumulative frequency (CF) ratio
WP	= working probit is (N + minimum PP) for each class
WC	= weighting coefficients is PP x number of samples

continued...

Plasma GH	Type 1 diabetes NR				
	CF	EP	PP	N	WP
minimum PP			3.1		
0.5	0		3.4	0	3.1
1.0	0		3.5	0	3.1
2.0	14.3	3.9	3.8	0.53	3.63
5.0	28.6	4.4	4.3	1.22	4.32
8.0	36.8	4.6	4.7	1.75	4.83
10.0	40.9	4.8	4.8	1.96	5.06
15.0	55.2	5.1	5.2	2.87	5.97
20.0	67.4	5.5	5.3	3.57	6.67
30.0	79.6	5.8	5.8	4.62	7.72
40.0	87.8	6.2	6.1	5.36	8.46
50.0	96	6.7	6.4	6.14	9.24
60.0	99.9	8.09	6.6	6.59	9.69
maximum PP	6.65				

Plasma GH	Control				
	CF	EP	PP	N	WP
minimum PP			3.7		
0.5	14	3.9	4.0	0.56	4.26
1.0	30	4.5	4.2	1.26	4.96
2.0	46	4.9	4.4	2.02	5.72
5.0	56	5.2	5.0	2.80	6.5
8.0	64.2	5.4	5.4	3.47	7.17
10.0	72.4	5.6	5.4	3.91	7.6
15.0	80.2	5.8	5.8	4.65	8.35
20.0	88.4	6.2	6.2	5.48	9.18
30.0	91.4	6.4	6.4	5.85	9.55
40.0	94.4	6.6	6.8	6.42	10.12
50.0	96.4	6.8	7.2	6.94	10.64
60.0	99.4	7.5	7.4	7.35	11.05
maximum PP	7.5				

Plasma GH	Type 1 diabetes EC				
	CF	EP	PP	N	WP
Minimum PP			3.3		
0.5	0		3.6	0	3.3
1.0	8.2	3.6	3.8	0.31	3.61
2.0	24.5	4.3	4.2	1.01	4.31
5.0	38.8	4.7	4.6	1.78	5.08
8.0	40.8	4.8	5.0	2.04	5.34
10.0	55.1	5.1	5.1	2.81	6.11
15.0	65.3	5.4	5.6	3.66	6.96
20.0	75.5	5.7	5.9	4.43	7.73
30.0	83.7	6.0	6.3	5.23	8.53
40.0	89.8	6.3	6.6	5.93	9.23
50.0	95.9	6.7	6.9	6.62	9.92
60.0	99.9	8.1	7.1	7.09	10.39
maximum PP	7.2				

Plasma GH	Type 1 diabetes PI				
	CF	EP	PP	N	WP
Minimum PP			3.9		
0.5	0		4.2	0	3.90
1.0	12.2	3.8	4.4	0.53	4.40
2.0	34.6	4.6	4.8	1.63	5.53
5.0	63.2	5.3	5.1	3.21	7.11
8.0	79.5	5.8	5.8	4.58	8.48
10.0	81.5	5.9	5.9	4.78	8.68
15.0	89.7	6.3	6.4	5.69	9.59
20.0	93.7	6.5	6.8	6.37	10.27
30.0	97.7	7.0	7.2	7.03	10.93
40.0	99.7	7.7	7.6	7.52	11.42
50.0			8.0		
60.0			8.5		
maximum PP	8.6				

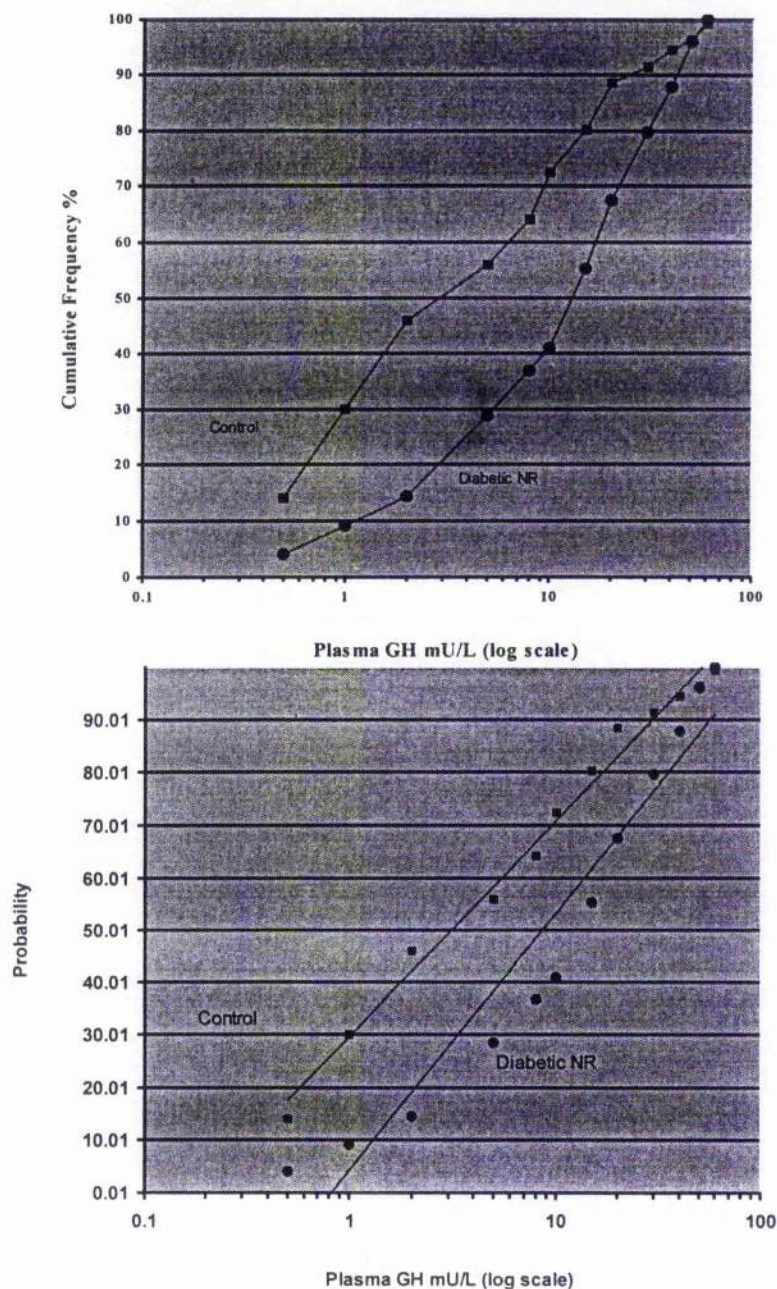


Figure 3c Performance: Cumulative frequency and Probability

Upper panel: The cumulative distribution (sigmoid curve) demonstrate graphically the differences in overnight GH concentration frequency between diabetic and control subjects. *Approximate* estimates of OC parameters can be made from such plots.

Lower panel: Transformation of percentages into probits (probability distribution) enables a robust estimates of OC parameters by regression analysis of all the data.

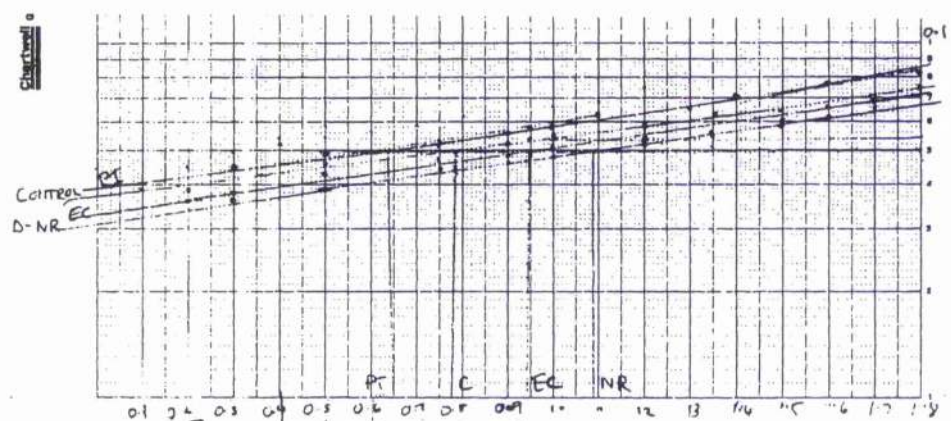
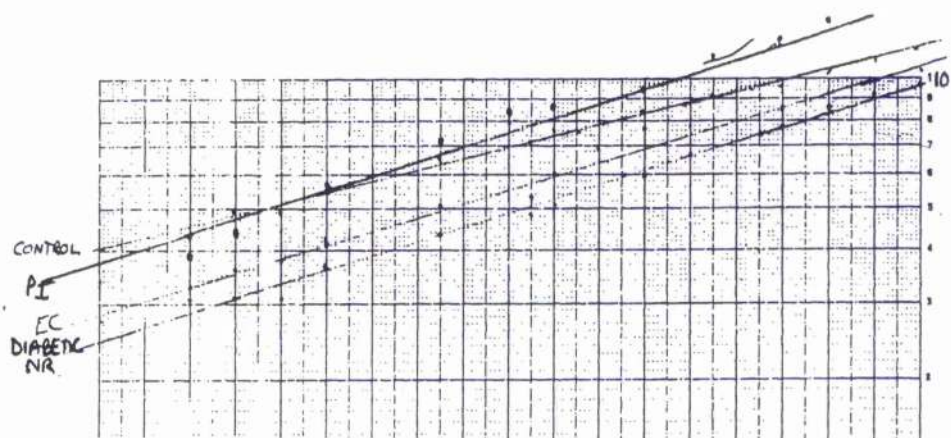


Figure 3d Probability plots (original data)

Lower panel: Using cumulative distribution analysis Empirical probits (EP) are derived from Fisher and Yates Tables. These values are plotted on Chartwell paper and Provisional Probits (PP) are read off at each log [GH] value. The minimum PP differs in each group.

Upper Panel: Working probits are calculated and plotted. Using Schaffler's 90% confidence intervals of regression lines, as the comparative probit lines do not overlap, they are different with a null hypothesis of $p < 0.0025$. The standard deviation of the observations can be calculated from the slope of the probit (as 5-95% encompasses $+2SD$)

For diabetic subjects (NR) OC50 (SD): 8.91 (2.69) mU/L
OC75 (SD): 28.61 (3.44) mU/L

These data were used for convolution.

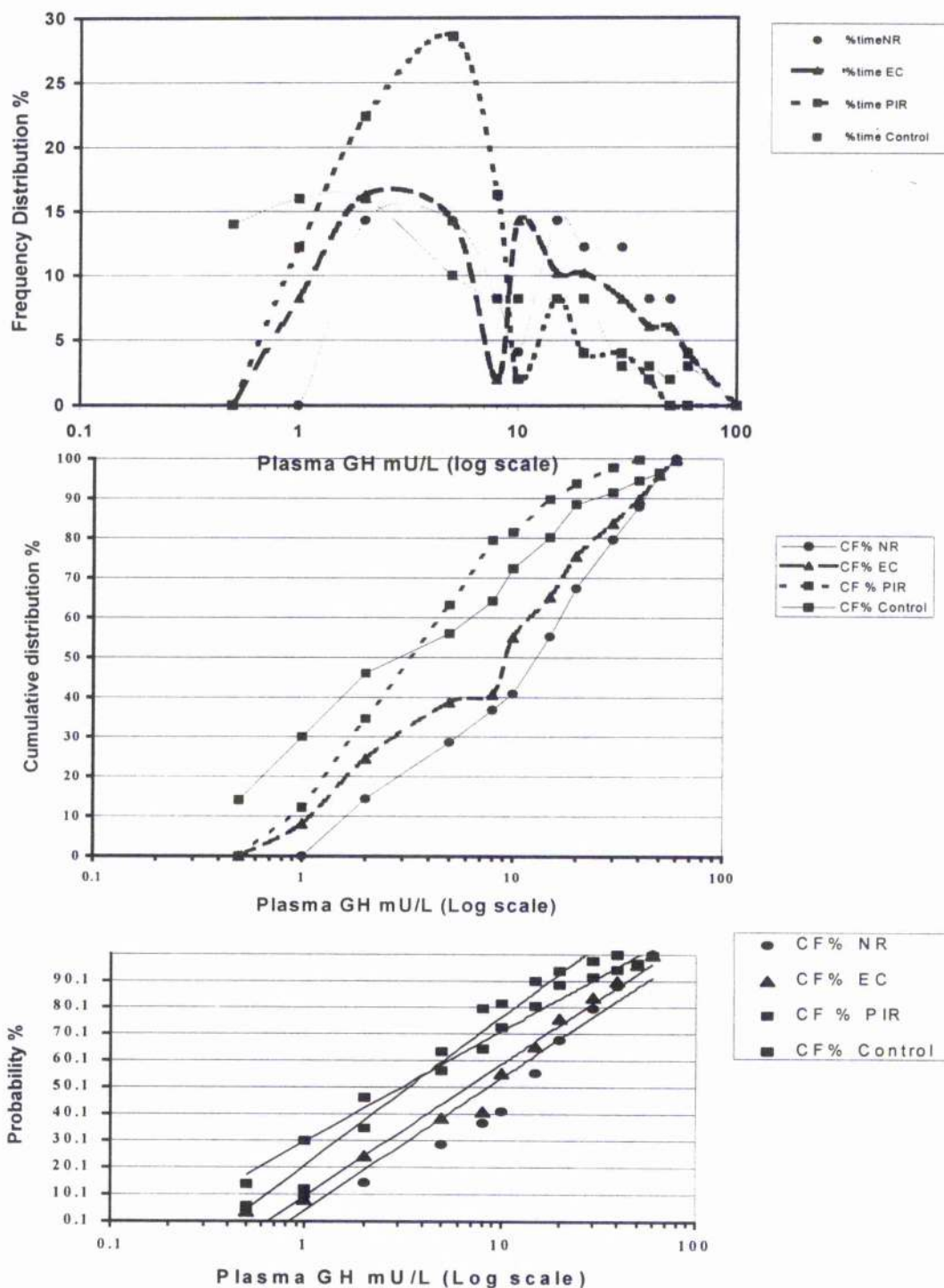


Figure 3e Comparison of all groups

Pooled data (all puberty groups) can be presented as a frequency distribution (upper panel), a cumulative frequency distribution (middle panel) and as a probability plot (lower panel). Pirenzepine (PIR) brings GH levels towards control values. Euglycaemia (EC) is similar to normal regime (NR).

Effect of puberty

The effect of stature and gender on trough GH has not previously shown to differ (Matthews 1991) but age influences this with a difference between young <9years having higher levels compared to older children >9years. The control data (plasma GH values for normal subjects in different stages of puberty) was pooled for analysis as all adolescent subjects were above 9 years. Puberty stage and gender do influence mean and peak GH levels but I have not quantified this separately by probit analysis.

Diabetes vs. Control

All GH profiles irrespective of puberty group was pooled. Diabetic subjects on normal regime insulin (NR), euglycaemic clamp (EC) and pirenzepine (PI) were compared to control subjects. The GH concentrations were calculated from Probit analysis. Parametric statistics were used to compare mean values. The confidence interval of the mean was used to compare values (95% CI, two-tailed α at $p < 0.05$). Confidence interval was calculated from mean $\pm 1.96 \times SD/\sqrt{N}$.

	Type 1 diabetes Plasma GH (95%CI) mU/L		Control Plasma GH (95%CI) mU/L		*p value
OC50	8.91	(7.83, 9.99)	2.23	(1.09, 3.37)	$p < 0.05$
OC75	28.61	(27.49, 29.82)	16.98	(15.89, 18.07)	$p < 0.05$
OC95	60.26	(59.12, 61.40)	25.41	(24.29, 26.53)	$p < 0.05$

*probability lines for whole series differ being outside CI with significance of $p < 0.0025$

Effect of euglycaemia

The effects of euglycaemia can also be compared to normal subjects and diabetics on normal insulin regime (Figure 4.3d).

Diabetic subjects	Normal regime plasma GH (95%CI) mU/L		Euglycaemic clamp plasma GH (95%CI) mU/L		*p value
OC50	8.91	(7.83, 9.99)	5.62	(4.46, 6.78)	p < 0.05
OC75	28.61	(27.49, 29.82)	21.37	(20.19, 22.55)	p < 0.05
OC95	60.26	(59.12, 61.40)	45.15	(40.34, 49.96)	p < 0.05

This suggests that during euglycaemia clamp GH occupies mean and peak dwell times at lower plasma GH than NR night. However, EC subjects still have significantly higher GH levels compared to control subjects (probability lines outwith 90% confidence). Interestingly, the lines of cumulative probability for diabetes diverge at high levels (**Figure 4.3d**). This reflects the grossly elevated peak GH (OC99) in these diabetic subjects (with levels of 70-90mU) double that seen in normal subjects (OC99 45 mU/L) during puberty.

The disparity between GH levels between control and diabetic subjects is related to different dwell times at all concentrations (**Table 4.3c**). In diabetic subjects the peak GH levels are 3.5 to 7 times the mean GH levels.

As the diabetic subjects have raised GH levels during both normal regime (NR) or clamp (EC) conditions, the respective findings for both groups of diabetic subjects was used :

Subjects with Type 1 diabetes	
Mean plasma GH (NR and EC groups)	
OC50 (mean 95% CI)	7.27 (6.14, 8.38) mU/L
OC75 (mean 95%CI)	24.99(23.84, 26.19) mU/L
OC95(mean 95%CI)	52.71 (49.73, 55.68) mU/L

Thus, the aim for reproducing a GH profile during puberty, would consist of peak pulse GH level between OC75 and OC95 (i.e. 24.99-52.71 mU/L. The difference between peak and mean level should be approximately 3.4 - 7.25 times in magnitude.

Effect of anticholinergic

After pirenzepine administration the diabetic subjects have reduced GH levels with mean (OC50) at similar GH levels seen in control subjects but with lower peak values (Probit Regression lines, **Figure 4.3d,e**).

(95% CI, two-tailed α at $p < 0.05$).

	Type 1 diabetes Pirenzepine plasma GH (SD) mU/L		Control plasma GH (95%CI) mU/L		*p value
OC50	2.24	(0.87, 3.61)	2.23	(1.09, 3.37)	NS
OC75	7.08	(5.66, 8.5)	16.98	(15.89, 18.07)	$p < 0.05$
OC95	15.14	(13.74, 16.54)	25.41	(24.29, 26.53)	$p < 0.05$

*No significant difference between probability lines as these overlap at 95% CI at mean and lower occupancies.

Trough levels

The OC5 level for subjects with Type 1 diabetes (NR 1.50, EC 0.85) and control 0.45 mU/L also differed significantly. The trough level in diabetic subjects was reduced after pirenzepine (PIR 0.7 mU/L). Matthews 1991 has estimated OC5 during puberty as being constant around 0.37-0.68 mU/L and in adult life as falling to 0.03 mU/L.

Type 1 diabetic subjects in puberty thus have higher minimum trough GH levels than expected in puberty.

4.0.8 GH secretion characteristics

The half-life data for GH used in the analysis of the GH profiles from the normal subjects was that determined by Hindmarsh, 1989, 1990. The GH half-life data employed in the deconvolution analysis of profiles from diabetic subjects were those from Chapter 3: - *Bolus half-life mean 13.6min (range 11.9-19.4); 60min exposure was 25.7min (range 19.4-37.1) and steady state (prolonged GH exposure) mean 28.5 (range 24.3-33.7) min.* The volume of distribution used

was 0.089L/kg in the diabetics (section 3.0.8) and 0.054L/kg in normals (Hindmarsh 1989).

Deconvolution analysis of GH profile data was analysed by the methods of Time Series analysis (Hindmarsh 1988b). Noise reduction was carried out by use of a three-minute moving average and the data was stationarised to remove any underlying long-term trends by linear regression. These stationary data arrays thus had no progressive shift in the baseline with time. Fourier transforms produced composite spectra of data showing all the dominant and sub-dominant harmonics together with an estimate of power and amplitude. The data can therefore be pooled using parametric statistics to yield spectra that are composite for the groups of data. A power spectrum displays the data as a histogram of power and frequency.

GH Secretory characteristics by Deconvolution

Figure 4.4b provides a schematic example of a deconvolution secretory profile. The raw data illustrating plasma profiles, variable half-life and deconvolution analysis of GH data comparing diabetic and control subjects by puberty group are shown in Appendix 6. The mean GH secretion is summarised in Figures 4.4c-g.

Diabetic subjects (NR) versus Controls

Mean overnight secretion ranged from 0.56-3.81 mU/min (median 1.88) in diabetic subjects: highest values were seen at puberty stage 4/5 in both boys and girls. In control subjects, mean GH secretion ranged from 0.32-1.92 mU/min (median 0.60): highest values were seen at puberty stage 2/3 in girls and 4/5 in boys. GH secretion was increased in diabetic adolescents compared with matched controls in all puberty groups and both sexes, $p < 0.001$ (Figure 4.4c).

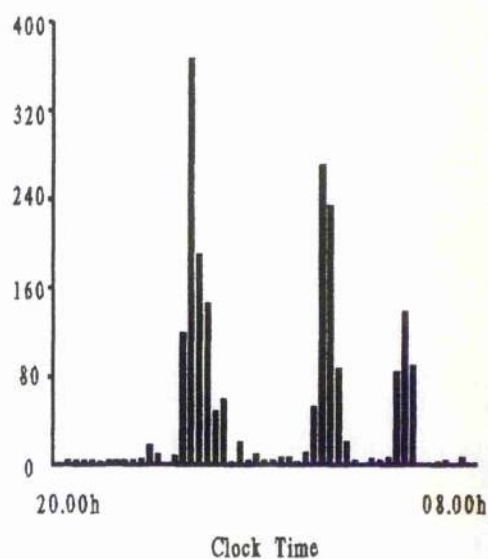
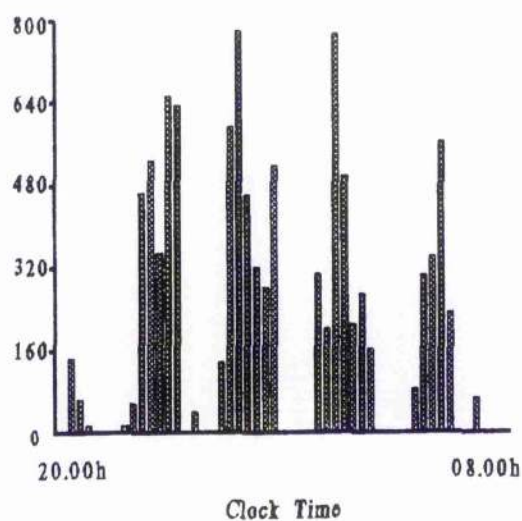
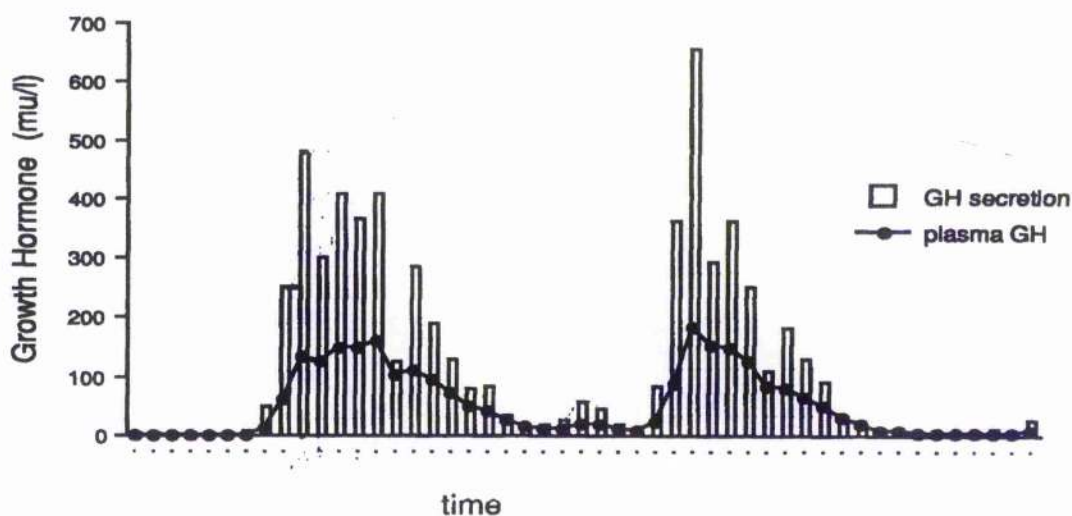


Figure 4a Performance of deconvolution to estimate GH secretion

Upper panel: Schematic representation of a plasma GH concentration profile and the calculated GH secretion

Lower panel: GH secretion (mU/h) in an adolescent subject with Type 1 diabetes (left) and in a puberty-matched control subject (right).

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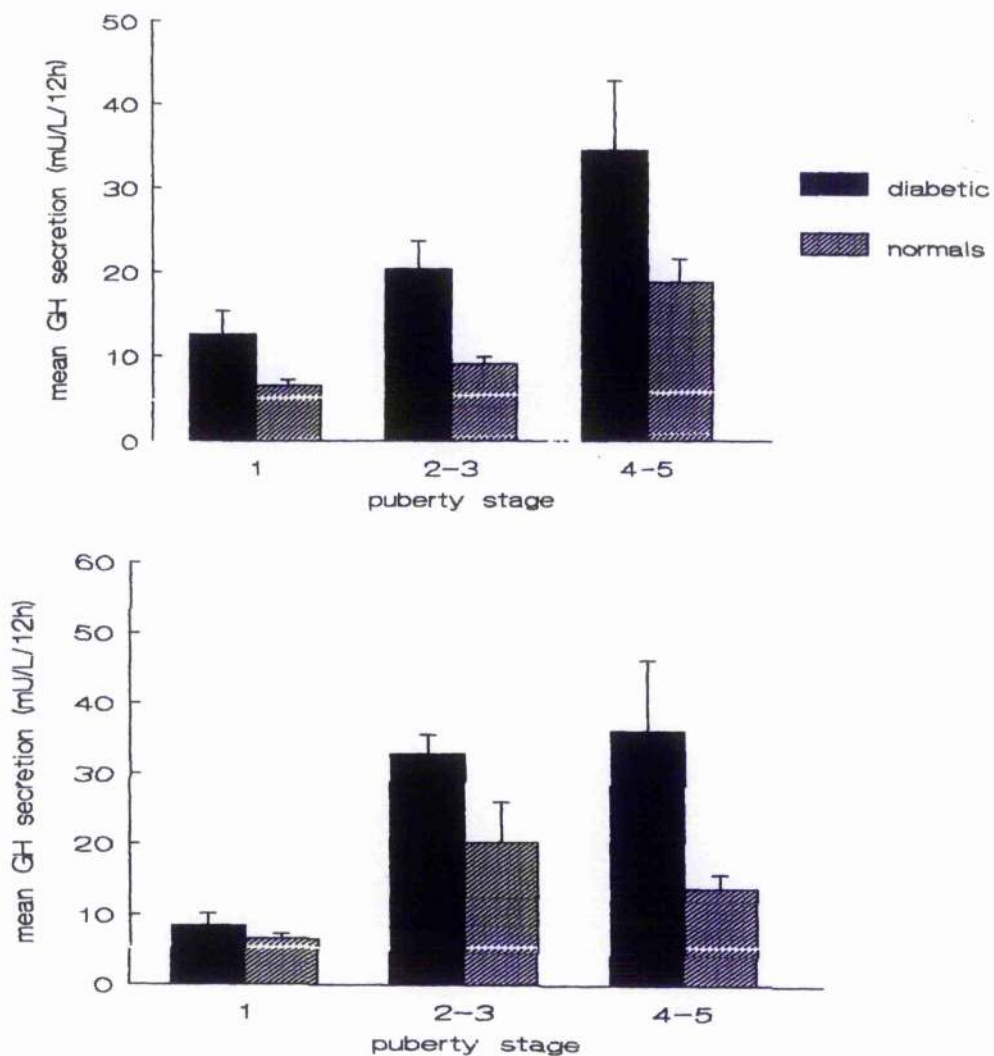


Figure 4b Summary mean overnight GH Secretion: diabetes versus control

Mean overnight secretion significantly differed ($p < 0.001$) between diabetic and control adolescents when analysed by sex or by puberty stage.

Upper panel: male lower panel: female

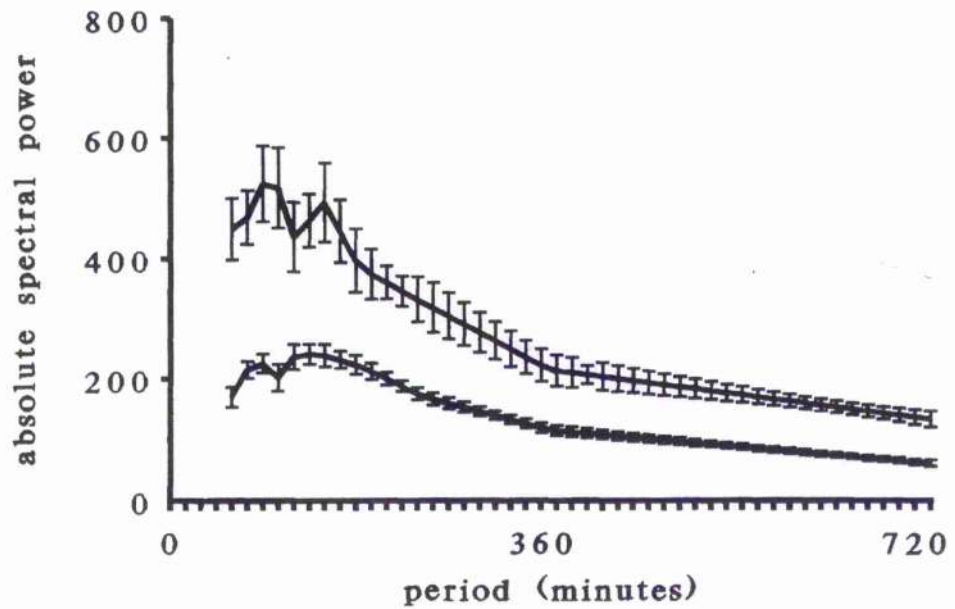


Figure 4c Periodicity of GH secretion: diabetes versus control

Spectral power of the Fourier transformation of overnight GH secretion in diabetic (upper spectrum) and normal adolescents (lower spectrum) pooling all puberty groups.

The dominant pulse periodicity (*maximum spectral power*) is shorter at 90minutes in diabetics, compared to 135minutes in normal adolescents ($p=0.0256$). The amplitude at all periodicities is greater in the diabetic group. Error bars show SEM

Periodicity

Visual scrutiny of the original plasma GH profiles revealed an average of 3-5 pulses over a 12-hour overnight period in diabetic subjects.

The Fourier transform of the GH secretory data demonstrated that the dominant pulse periodicity for GH secretion in diabetics was shorter at 90 minutes, when analysed by sex or puberty stage, compared to that found in control (normal subjects) at 135 minutes $p=0.0256$ (Figure 4.4d). The amplitude of GH pulses at each periodicity was greater in the diabetic group.

Euglycaemia and Pirenzepine – effect on GH secretion

Deconvolution analysis in the 29 subjects who had overnight euglycaemic clamp showed no significant difference in GH secretory characteristics from the 15 subjects during normal insulin regime (NR) night (Figure 4.4e,f). The mean overnight GH secretion rates were similar (NR 1.82 ± 0.1 versus EC 2.04 ± 0.25 mU/min) except in 4 boys at stage 4-5 where there was a noticeable increase during euglycaemic clamp from 2.29 ± 0.14 to 3.92 ± 1.05 mU/min. The dominant pulse periodicity remained at 90min during euglycaemic clamp.

Pirenzepine administration ($n=7$) reduced the mean overnight GH secretion in Type 1 diabetes to levels which were comparable with the normal children. The mean GH secretion in seven diabetic subjects on EC compared to EC+pirenzepine was reduced from 1.59 ± 0.19 to 0.71 ± 0.08 mU/min $p<0.001$. Expressed as a percentage GH secretion was reduced by 9.3 - 82.8% (median 63%) compared to respective subjects' clamp night (Figure 4.4g).

Fourier analysis confirmed that GH pulse amplitude at each pulse periodicity was reduced but the dominant periodicity was maintained at 90min.

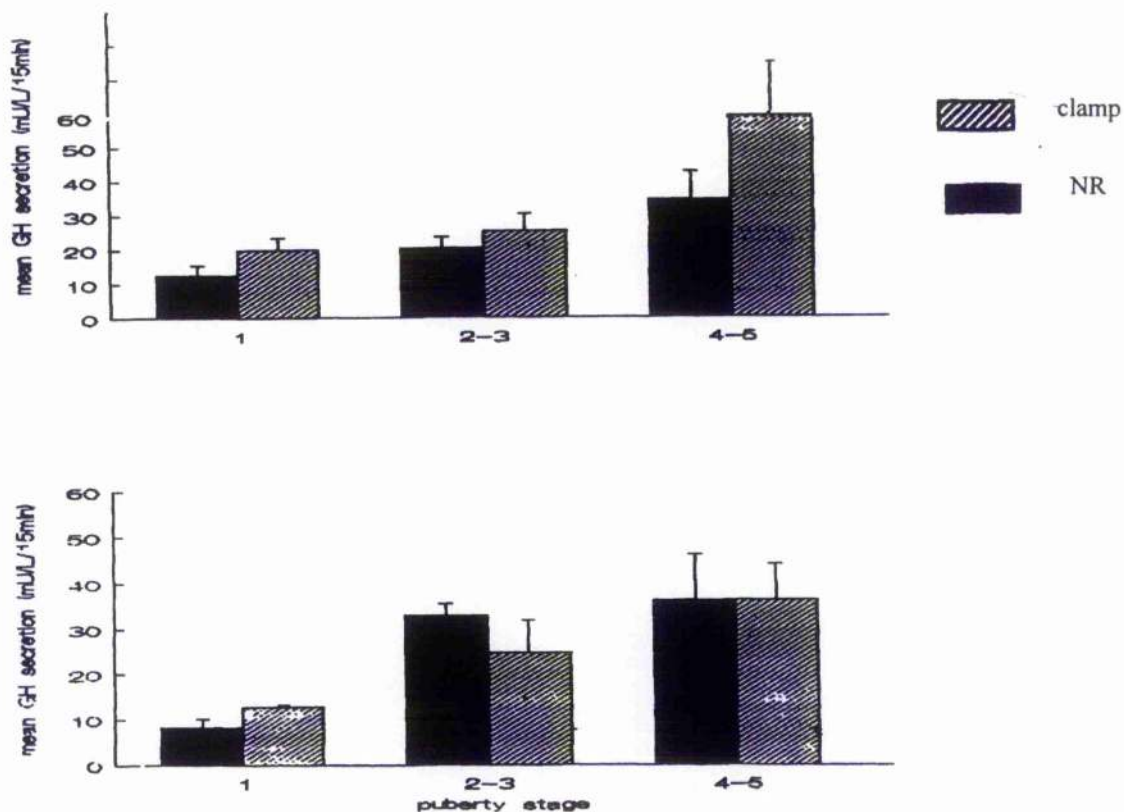


Figure 4d GH secretion in Diabetics: Normal insulin regime versus subjects' euglycaemic clamp

This shows that there was no significant difference in GH secretion between NR and clamp nights apart from in puberty group 4/5 in boys.

Upper panel: Male Lower panel: Female

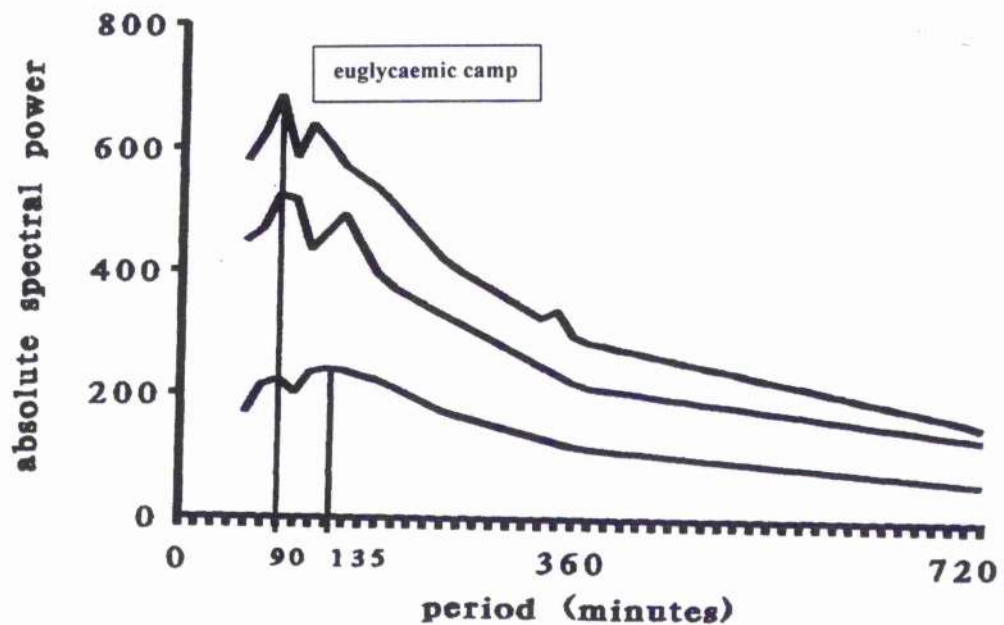


Figure 4e Pulse periodicity in diabetic subjects: normal insulin regime and euglycaemic clamp

Upper spectrum: Overnight euglycaemic clamp (EC) did not alter maximum GH pulse periodicity (top line) which remained at 90 minutes, similar to subjects' NR night

Lower spectrum: Control subjects with maximum pulse periodicity at 135minutes.

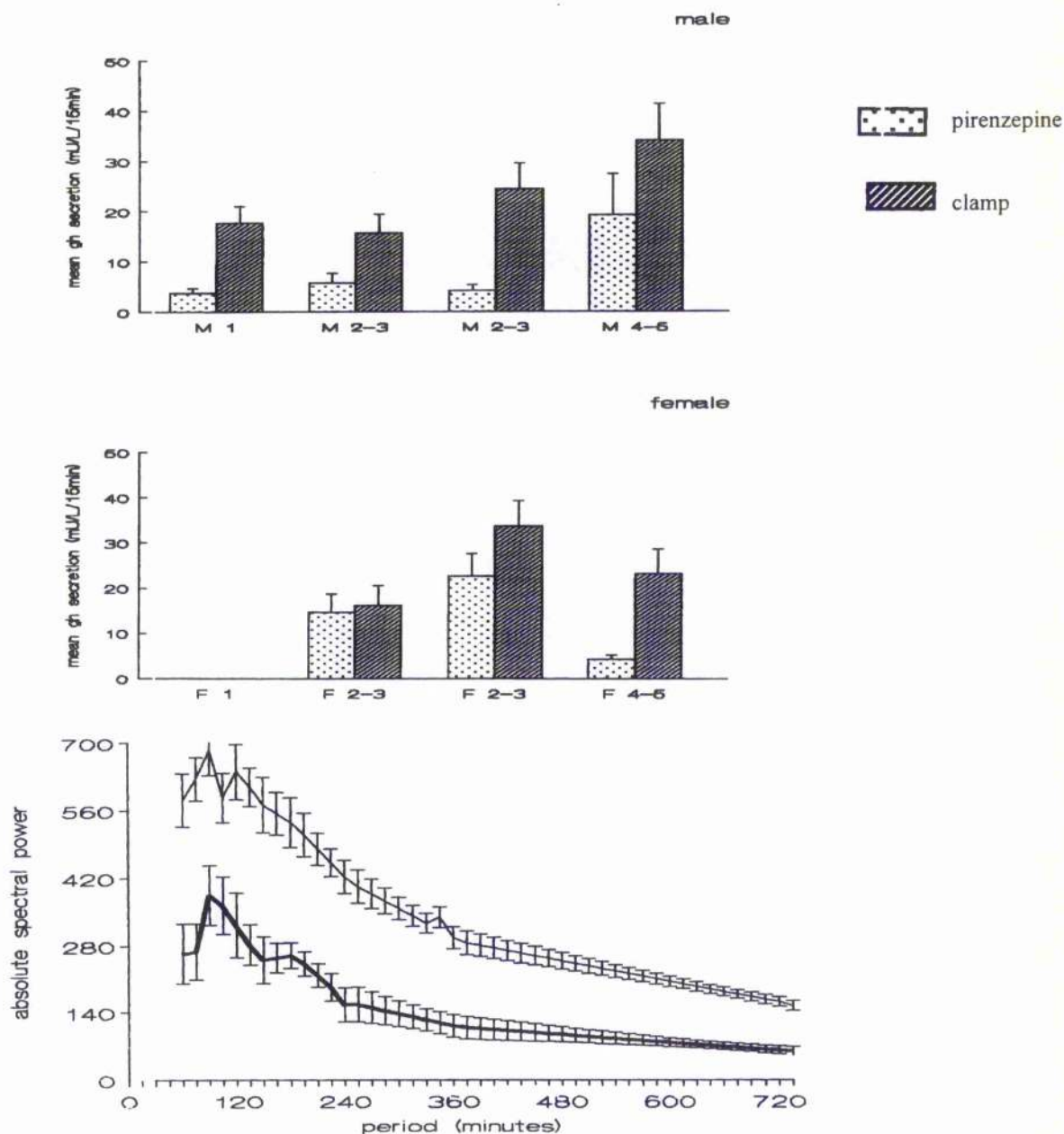


Figure 4f Secretory rates in diabetic subjects: effect of pirenzepine (anticholinergic) versus euglycaemic clamp (EC)

Upper panel: Pirenzepine reduced the mean overnight GH secretion in all puberty groups compared to subjects' clamp night **Lower panel:** spectral power. The dominant pulse periodicity was maintained at 90minutes although the amplitude was reduced at all periodicities

4.0.9

SUMMARY AND DISCUSSION

The development of objective, statistically based, and reproducible computerised algorithms to quantify episodic GH release is numerous (Urban 1988). Three basic approaches have been discerned: peak detection, time series analysis (frequency spectra) and modelling.

Originally Santen and Bardin defined a secretory pulse as 'an increment from nadir to peak of greater than 20%'. Later modifications, by other authors, allowed for within-assay noise (non-systematic fluctuation) and defined the pulse as an increment of x times the intra-assay coefficient of variation and x was between 3 and 5. Even with modifications that there was a high false positive rate, which was worst at high sampling frequencies (Royston 1989). Urban (1988), in a detailed comparative study of eight computer programs for pulse detection found that the Type 1 error rate increased with the integrated area under curve (IAC). The methods described previously (Merriam & Watcher's PULSAR algorithm, Clifton & Steiner's 'Cycle Detector') all have disadvantages. The methods of Van Cauter (1981) and Veldhuis & Johnson (1986) emerged from the study of Urban, (1988) as having similar performance, judging by a concordance of 70-80% between number of peaks detected and as having well controlled Type I error rates. The method of Clayton (1987) uses a simple iterative method of trial peaks (governed by two parameters-a mean and a minimum height peak), its Type I error can be controlled satisfactorily but requires further comparative testing. A major problem with peak picking is the dependence of the number of pulses found on the sampling interval employed. The number continues to rise even at 5min sampling. Thus studies which use different sampling regimes are hard to compare. Another problem is that the expression of results as means of peak heights, interpeak intervals may be misleading if the standard deviations of these quantities are

large. It is therefore important that the researcher specifies clearly the questions he or she is trying to answer and selects sensible summary statistics accordingly.

In this chapter overnight plasma GH profiles have been analysed by an objective method to describe characteristics in a simple and robust manner. The Distribution Method of analysis allows an objective "best estimate" when reconstructing a "physiological" GH profile. This GH profile can be separated into its components of minimum (OC5), mean (OC50) and peak (OC75 or OC95) which describes the "dwell-times" at "observed concentrations". As the distribution data is pooled GH levels seen in diabetic subjects at all stages of puberty, the analysis reflects the GH levels experienced by most adolescent diabetics. Diabetic subjects on their normal insulin regime and during euglycaemic clamp have significantly higher GH levels to puberty-matched control subjects. Pirenzepine administration to diabetic subjects brings GH OC parameters towards that seen in control subjects.

Analysis of trough levels may be particularly important in understanding the regulation of GH secretion and action. The metabolic significance of the "height" of the baseline of hormone concentrations is likely to be related to receptor or post-receptor 'recovery' characteristics since up-regulation and down-regulation are time dependent phenomena. I have therefore utilised this data to model a continuous and pulse GH profile in order to examine the signal for metabolic disturbances (Chapter 5).

In order to describe GH secretion in adolescent subjects, deconvolution analysis of overnight (20.00-08.00h) of GH profiles was performed. I have used the model proposed by Hindmarsh, 1990 (with the measured variable half-life and volume of distribution in normal subjects and that measured in diabetic subjects (Table 3.2f). This model is preferred to that suggested by Veldhuis, (1987) and others (Appendix 1b) since this model utilises an accurate assessment of the circumstances that affect the clearance characteristics in the study

subjects. I have shown that in diabetic adolescents pituitary GH secretion is increased (mean and amplitude of secretion) at all puberty stages and in both sexes compared to matched controls. A shorter dominant pulse periodicity of secretory episodes (90 minutes compared to 135 minutes in normal subjects) suggests the frequency of GH secretory episodes is also increased.

Neither GH secretory amplitude nor frequency is affected significantly by overnight euglycaemic clamp. The anticholinergic drug, pirenzepine appears to suppress GH pulse amplitude secretion but has no effect on frequency of GH secretion. Cholinergic muscarinic receptor blockade with pirenzepine suppresses GH release in normal subjects and in patients with Type 1 diabetes and is thought to act via SRIH release. This suggests that the mechanism of hypothalamic regulation of GH release in Type 1 diabetes may be abnormal with a persistent existence of reduced somatostatin suppression due to cholinergic overdrive.

Some authors have found a marked difference in GH secretion with increased levels accentuated in prepubertal subjects, but during puberty in boys, there was little difference in GH secretion compared to normal subjects (Nieves-Rivera 1993). My analysis of GH profiles in diabetic adolescents on their usual regime NR, suggests that there was increased mean overnight GH secretion at all puberty stages compared to normal subjects ($p < 0.001$) (Figure 4.4c (median (range) GH secretion: diabetics NR and EC was 1.88 (0.56-3.81), control group was 0.62 (0.32-1.92) mU/min).

In suboptimally controlled adults with Type 1 diabetes there was altered pulsatility (Asplin 1989). This is confirmed in my adolescent subjects where the dominant pulse periodicity was altered (diabetics 90min, control 135min, $p < 0.0256$). Interestingly, this intrinsic 90 min rhythmicity of GH release in diabetics is also seen in normal adult subjects during fasting. When comparing a control fed day with the first and fifth days of a five day fast, GH secretion changes were accompanied by both frequency and amplitude modulation of the pulsatile GH secretion (Ho 1988, Hartman 1992, 1996). Furthermore, as there

was also an uniformity of a 90minute cycle on both fasted days; this suggests the presence of an intrinsic 90-minute ultradian rhythm associated with the release of GH in man. A number of behavioural and neuro-muscular functions in man have a similar cycle of occurrence these include rapid eye movements sleep (Holl 1991), dreaming and tumescence, blood pressure variations.

In contradiction, animal studies in the fed state suggested the presence of frequency components of 3-5 hourly pulses of GH which were postulated to be meal cued events (Driver 1981). However, during sampling studies carried out in the fasting state, these investigators noted an enhancement of pulse frequency associated with new frequencies. This three-hour periodicity appears to be a harmonic of the more fundamental 90-minute rhythm identified during the physiological adaptation to starvation.

It is also possible that I may have under-estimated the number of GH secretory episodes: more frequent sampling of 1min intervals (compared to 20min) may detect twice as many pulses (Holl 1991); and ultrasensitive GH assays (sensitivity 0.002 $\mu\text{g/L}$) (Iranmanesh 1994, Veldhuis 1995) may allow better recognition of the neurosecretory pattern of GH secretion (Winer 1990, Reutens 1996, Veldhuis 1996c). However, observations of my GH data, using integrated sampling techniques which would be less likely to miss a pulse, do suggest a difference in pulse frequency between the two puberty-matched groups (control versus diabetic subjects).

In subjects with Type 1 diabetes, short-term euglycaemia does not reduce the parameters of GH secretion. During overnight euglycaemic clamp, neither mean GH secretion nor dominant pulse periodicity showed any significant difference from subjects' normal insulin regime night (mean \pm SEM: overnight GH secretory rates EC 1.82 ± 0.33 versus NR 1.91 ± 0.37 mU/min, $p=\text{NS}$ by Student's paired t-test); dominant pulse periodicity 90min. This has also been shown in clinical studies where the spontaneous GH release in Type 1 diabetes patients could not be restored to normal despite maintenance of improved control during the periods of either intensified conventional insulin

treatment or continuous subcutaneous insulin infusion (Vigneri 1976, Arias 1984, Hermansen 1987).

The shorter pulse periodicity in diabetic subjects suggests either that the periods of SRIH withdrawal and/or GHRH secretion may be abnormally frequent. It has been reported that GH release can occur at higher blood glucose concentrations in Type 1 diabetes than in normal individuals (DeFronzo 1980), suggesting that oscillations of blood glucose, even without frank hypoglycaemia, may result in GH secretion. Others have shown that fluctuations in blood glucose concentrations during sampling period explained some but not all, of the GH secretory episodes (Molnar 1972).

The possible mechanisms for increased GH secretion must still be explained by events at the pituitary or hypothalamic level. The fact that the GH cannot be normalised by good metabolic control (euglycaemia) suggests that the increased GH release may be due to a hypothalamic or pituitary dysfunction induced either by the diabetes itself or by the treatment of the diabetes.

SRIH plays a major role in GH regulation and previous evidence suggest that decreased hypothalamic SRIH secretion in Type 1 diabetes is likely to be responsible (Schaper 1992). The mechanisms of metabolic interaction on hypothalamic SRIH are unclear.

It has been suggested that glucose may directly suppress somatostatin release at the hypothalamus in rats (Lewis 1987) however, in normal man, hyperglycaemia suppresses GH secretion, presumably by increasing hypothalamic somatostatin secretion (Penalva 1989). This suppressive effect of hyperglycaemia appears to be independent of the concomitant increase in plasma insulin (Schaper 1990). As hyperglycaemia does not inhibit GH secretion in response to various stimuli in Type I diabetes (Press 1984b, Ajlouni 1975), this suggests a defect in hypothalamic SRIH regulation of GH secretion: a reduced somatostatin (tone) in Type 1 diabetes allowing increased GH secretion at higher blood glucose concentrations.

The high GH concentrations may be a direct result of feedback from low levels of IGF-1 in some studies of Type 1 diabetes (Lanes 1985). In some hyperglycaemic studies, the serum IGF-1 level was noted to be depressed in diabetic subjects in comparison to healthy volunteers (Schaper 1990) suggesting that IGF-1 may also be a contributory factor of the effect of glucose on GH secretion in Type 1 diabetes. The inhibitory effect of IGF-1 at the hypothalamic level is felt to be mediated by both inhibition of GHRH and stimulation of somatostatin (Berelowitz 1981), but other studies have shown that IGF-1 acts on a different population of somatotrophes from somatostatin (Hoeffler 1987). Since IGF-1 suppresses pituitary response to GHRH (Berelowitz 1981) the low IGF-1 levels in diabetes could explain the exaggerated responses to this and other stimuli. However, several studies on strictly well-controlled diabetic subjects ($HbA1 < 6.5\%$) show that an increased GH response to GHRH-stimulation still persisted while IGF-1 levels were normalised to that comparable to control subjects (Schaper 1990), suggesting that either the reduced IGF-1 is not the major cause of increased secretion; or that the changes in IGF-1 binding proteins could alter its feed back on GH secretion (Baxter 1987, Suikkari 1988); or that there is a disturbance in the short-term autoregulation of GH secretion (i.e. GH stimulating hypothalamic somatostatin) (Ross 1987); or other metabolic factors are involved.

The mechanism of GH suppression by anticholinergic agents is also speculative but may be related to the tonic inhibitory effect exerted by acetylcholine on endogenous SRIH release from the hypothalamus (Richardson 1980, Goni 1997). Cholinergic blockade thus releases endogenous SRIH that in turn inhibits GH release from the anterior pituitary. Results from this chapter have confirmed that pirenzepine does suppress GH secretion by reducing overall secretion (mean \pm SEM GH secretion from 1.57 ± 0.19 to 0.71 ± 0.80 mU/min, $p < 0.001$ showing a median (range) reduction of 63% (9.3-82.8%) when compared to the subjects' clamp night) with reduction in pulse amplitude at all secretory levels to nearer that seen in normal subjects. Curiously

pirenzepine has no effect on the periodicity of GH pulse secretion which remained at 90min (135min in normal subjects) in diabetic subjects during either their usual insulin regime(NR) or euglycaemic clamp (EC).

This suggests that the primary abnormality of GH secretion in Type 1 diabetes may be caused either by GHRH alterations or that pirenzepine may cause an increase in SRIH tone which may be sufficient to reduce the amplitude of the GH secretory episode in response to GHRH pulses but insufficient to prevent response to every GHRH pulse stimulus.

Pietschmann (1986) and Ismail (1993) confirm that cholinergic muscarinic blockade with pirenzepine greatly suppresses GH responses to maximal stimulatory dose of GHRH in both normal and diabetic adult subjects but the GH response was still significantly higher in diabetic subjects. This is consistent with a decreased sensitivity to muscarinic cholinergic receptor blockade (Coiro 1990). Interestingly, enhancement of cholinergic tone by pyridostigmine causes enhanced GH responses to GHRH in normal subjects (Massara 1986, Ross 1987, Penalva 1990), but in contrast did not lead to enhancement of GH response in diabetic subjects (Ismail 1993). Diabetic subjects also appeared to have a similar level of GH response to GHRH as normal subjects with additional pyridostigmine (Ishmail 1993). In addition, experimental diabetes in rats is associated with significant alterations in the brain cholinergic system (Wahba & Soliman 1988) providing further evidence for the importance of this system in diabetes. These data suggest that the level of hypothalamic cholinergic tone in diabetic subjects is similar to the enhanced cholinergic tone seen in normal subjects pre-treated with pyridostigmine.

There is accumulating physiological evidence that, in addition to the opposing actions of GHRH and SRIH on anterior pituitary cells, SRIH can also exert a tonic inhibitory influence on hypothalamic GHRH secretion. Evidence in Type 1 diabetic subjects suggests that impaired somatostatin tone exists during hyperglycaemia whereas a normal GH response to GHRH was seen (Martina 1997). Furthermore, GH negative feedback on its own secretion

occurs predominantly through increased hypothalamic somatostatin secretion, which is under inhibitory cholinergic control (Ross 1987) so that this mechanism of auto-feedback would be deranged in patients with diabetes.

It appears that although modification in hypothalamic neuropeptides, GHRH and SRIH, may be altered in Type 1 diabetes, changes in the sensitivity of the hypothalamus and pituitary to the feedback effects of GH may also occur. Recent work in poorly controlled diabetic rats suggest that the expression of both GHRH and SRIH declines specifically in the anatomical areas involved in anterior pituitary control and further alterations of pituitary GH receptor mRNA occur with prolongation of diabetes (Busiguina 2000).

Summary

In adolescents with Type 1 diabetes on their normal insulin regime, pituitary GH secretion is increased at all puberty stages and in both sexes compared to matched controls. During GH secretion, dominant pulse periodicity, by Fourier analysis, is shorter at 90min in Type 1 diabetes (135min in normal subjects). Euglycaemia does not alter secretory characteristics; the anticholinergic pirenzepine reduces the quantity secreted but not the frequency of pulses.

It appears therefore, that in diabetic subjects a reduced somatostatin tone is present because of cholinergic overactivity. This may persistently exist due to alterations in the expression of SRIH or the pituitary GH-receptor that is induced by diabetes itself or by the treatment of diabetes with insulin. I have examined the influence of insulin and SRIH on GH suppression and release in the next chapter.

CHAPTER 5

REGULATION OF GROWTH HORMONE CONTROL

5.0.0

INTRODUCTION

Somatostatin (SRIH) is known to inhibit basal and GHRH-stimulated GH secretion (Vance 1985) and previous studies defined withdrawal of hypothalamic somatostatin as the only involvement with the timing and duration of the GH pulse, whereas GHRH was responsible for the amplitude of the pulse. However, conflicting evidence still appears: somatostatin has been shown to decrease both the mass and frequency of GH secretory bursts (Calabresi 1996) and GHRH shown also to participate in the generation of GH pulses in humans (Jaffe 1993). Thus, the elevated levels of GH in Type 1 diabetes could arise because of decreased pituitary sensitivity to SRIH, decreased hypothalamic SRIH secretion, or increased pituitary sensitivity to GHRH.

There is still no consensus on the role of glucose and insulin in the regulation of GH secretion in Type 1 diabetes. The increase in GH frequency in the diabetic group suggests that the periods of SRIH withdrawal and/or GHRH secretion may be abnormally frequent. It may be that GHRH pulses are occurring with the same frequency in both groups, but that in the presence of reduced SRIH 'tone' in Type 1 diabetes allows more of these stimulatory pulses to result in pituitary GH secretory episodes. In this chapter, I have therefore analysed the effects of glucose and insulin on SRIH suppression of GH and on the endogenous GHRH-driven GH rebound secretion after ceasing SRIH.

Details of subjects with Type I (insulin-dependent) diabetes mellitus who attended for GH clearance study (section 2.0.4, Table 2.1a) and GH signal studies (section 2.1.4) are provided elsewhere. The study protocols are summarised in Chapter 2 and Figures 2.1a,b and detailed in section 2.0.5 and 2.1.5-2.1.9.

5.0.1 Analysis of interactions of somatostatin and insulin

Somatostatin is widely used in experimental metabolic studies to control hormone actions. Apart from the well-known suppressive effects, it has been suggested that somatostatin per se may alter insulin sensitivity. Previous studies suggest that the interaction of SRIH and insulin is time dependent (Adamson 1982). In the first hour of SRIH administration, this peptide is thought to potentiate the effect of insulin (Gerich 1979). Moller (1995) in forearm studies in normal volunteers, has shown that when somatostatin (dose of 25 μ g/h for 1 hour) is infused either as a systemic venous or local intra-arterial infusion during a hyperinsulinaemic euglycaemic clamp, enhanced insulin-stimulated glucose uptake occurred but only during local forearm infusion (55% greater compared to intra-arterial administration). Moller suggests that this insulin-stimulated muscle utilization of glucose could potentially interfere with metabolic studies, although the local mechanism remains unidentified. Other authors have shown that SRIH counteracts insulin after 2 hours (Lins 1976). The mechanism behind this anti-insulin action of SRIH whether it is localised to the liver or periphery or attributed to the antagonism of or resistance to the action of insulin still remains to be explained. These findings highlight that before any observations affecting alterations in insulin, glucose and GH dynamics are made, the effects of SRIH and insulin interactions require further clarification to ensure this is not a confounding factor.

I have examined the detailed changes and function of the insulin-varying clamp during GH signal studies. These subjects had a constant 13 hour infusion of SRIH (50mcg/m²/h). Subject H had been excluded as she had developed profound hypoglycaemia during initial clamp stabilization. Therefore a glucagon infusion at 1ng/kg/min was started at 1900h for all other subjects attending GH signal studies. Data from the GH signal study (SRIH+glucagon \pm GH) during a 12hour insulin-varying clamp were initially examined for clamp bias or drift and were compared to reported insulin-varying clamp data in Type 1 diabetic subjects without SRIH or glucagon

(Matthews 1990). The first 60minutes of the clamp during GH clearance study (without glucagon or GH administration) were also examined.

- | | |
|----------------|---|
| Group 1 | GH signal (control night)
6 diabetic subjects: SRIH+glucagon |
| Group 2 | GH signal (pulse and infusion)
10 diabetic subjects: SRIH+glucagon + GH
(the total dose of GH was 18 mU/kg). |
| Group 3 | Overnight clamp (Matthews 1990)
26 diabetic subjects without SRIH |
| Group 4 | GH clearance study t30-90 |

Clamp analysis

Bias: The bias of the glucose clamp theoretically depends on the insulin (infusion) clearance between subsequent periods glucose levels (G1 and G2).

For insulin-varying clamp this would be:

$$\text{Insulin clearance} = \frac{\text{insulin infused per unit time}}{\text{insulin concentration}}$$

Bias can be calculated as the difference in glucose (G2-G1) from the mean glucose (G1+G2/2).

The median coefficient of variation in the last 2 hours of clamp was calculated comparing consecutive time periods as

$$\text{CV \%} = \text{SD} \times 100 / \text{mean glucose}$$

5.0.2 Glucose and insulin on GH suppression and rebound secretion

The GH clearance study (section 2.0.2-6) allowed analysis of clamp stabilization (t30-90min) [SRIH + insulin + glucose]; and SRIH suppression on endogenous GH (t90-150min); and endogenous GH rebound (t150-270min). GH secretion was suppressed by somatostatin infusion starting at 08.30h (t=30) and terminated at t=150minutes. The insulin/glucose clamp was maintained for

the whole study ($t=270$ minutes) to generate normoinsulinaemia or hyperinsulinaemia (section 2.0.5). Growth hormone, glucose and metabolites were measured as described in Chapter 2 and Appendix 3b.

GH suppression and rebound analysis

Results are given as mean \pm SEM unless otherwise stated. Blood glucose was normally distributed. Linear interpolation with log-transformation normalised the GH and plasma free insulin data and therefore parametric statistical tests have been used.

Student's paired t-test was used to compare the data profiles from the same subject during the three clamp study mornings and unpaired t-test for whole group data, $p < 0.05$ was considered significant.

Correlation regression analysis was performed to examine the relationship between GH parameters (log₁₀ suppression or peak and mean rebound) and concurrent, previous hourly and mean plasma free insulin and glucose levels for an appropriate time period. Spearman's rank correlation was used to measure the degree of association between GH parameters and subjects' characteristics.

Confidence Intervals were used to describe GH suppression levels during different insulin and glucose levels.

5.0.3

RESULTS

5.0.4 Effect of somatostatin on insulin action

The initial blood glucose before commencing clamp in overnight studies was similar between Group 2 and Group 3. The interactions between glucagon and SRIH and insulin have been discussed in section 2.2.7 and accepted that at low glucagon levels, the superior effect of insulin can be seen (Paolisso 1987). **Table 5.4a** and **Figure 5.5a** displays characteristics of the respective clamp. Plasma free insulin concentrations are provided in **Table 6.5a** and insulin infusion rates during overnight clamp are demonstrated schematically in **Figure 5.5b**.

The maximum declination (dG/dI) was at 15 minutes for all study groups. Between 01.00-08.00h of all clamps the mean blood glucose achieved adequate euglycaemia (stable clamp period). Mean insulin infusion rates required for euglycaemia during the steady state period (01-0800h) ranged from

Group 1 SRIH+glucagon : 2.4-9.2 mU/kg/h (median 4.6)

Group 2 SRIH+glucagon + GH: 3.5-15.6 mU/kg/h (median 7.6).

This is significantly lower than those values reported previously by Matthews 1990 (5.3-23.3, median 14.3 mU/kg/h) without SRIH.

As it has been observed that during the first hour of SRIH administration, the peptide potentiates the effect of insulin, the function of clamp with only SRIH during the GH clearance study was scrutinized further. During GH clearance study SRIH infusion (started at $t=30$ mins) caused glucose levels to fall dramatically within 15-30mins of being commenced ($t=30-60$ min) causing the greatest rate of fall of glucose at 15min (HI clamp), 20mins (NG clamp) and 30mins for HG clamp. (The maximum effect of insulin on glucose declination occurs normally at 15mins). The change in glucose per unit time (dG/dt) during the first 60minutes ($t30-90$) of the three clamp conditions (NG,HI,HG) when

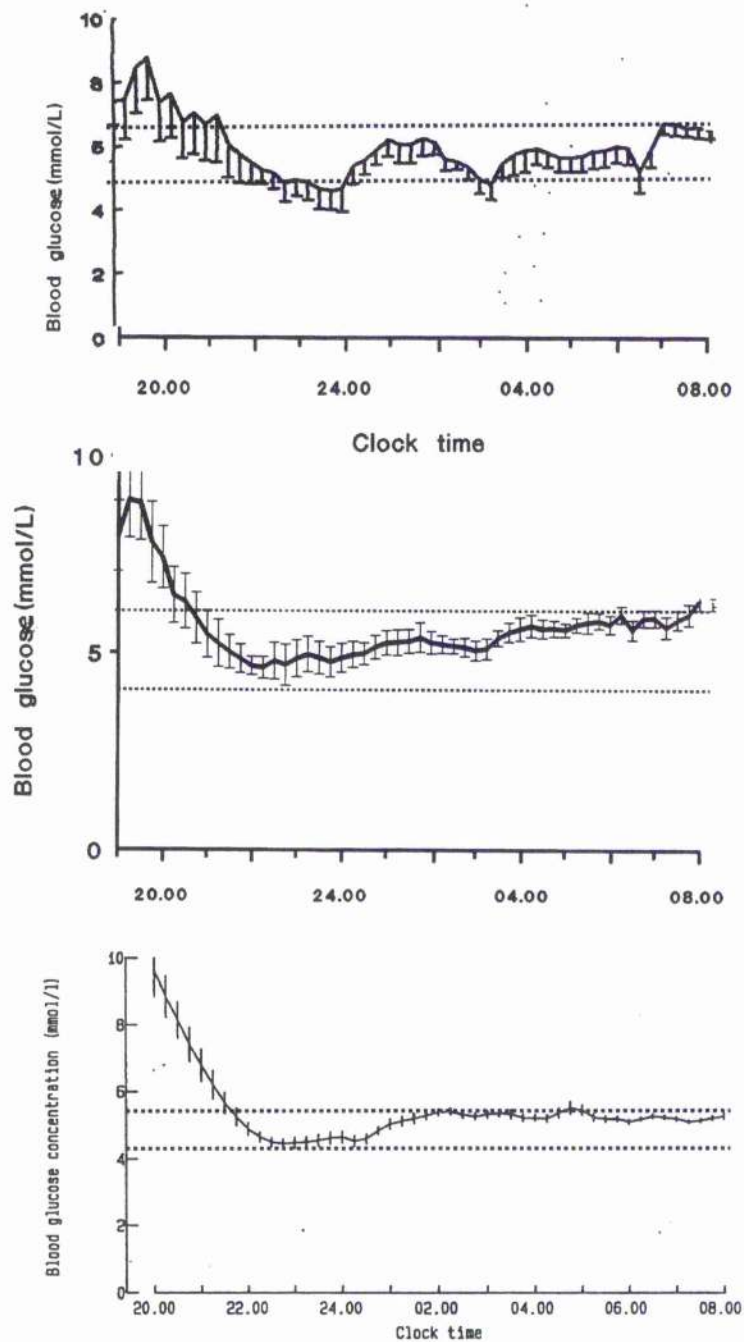


Figure 5a Overnight Glucose clamp with and without SRIF: Upper panel :group 1 SRIF without GH **Middle panel :** SRIF with GH **Lower panel:** group 3 Data from Matthews et al 29 subjects who did not have SRIF. To achieve target range glucose 5mmol/L the clamp must function under tight limits range (dashed lines).

insulin infusion was maintained at a constant rate to generate either normoinsulinaemia or hyperinsulinaemia (section 2.0.5).

Clamp changes during the first 60minutes of commencing SRIH (Figure 5.5c) can be described by the relationship

$$dG/\log PI = -0.1467(\log GI) + 3.326$$

where dG is the rate of change of glucose mmol/min

logPI is the log₁₀ plasma insulin

logGI is the log₁₀ glucose infusion rates (ml/kg/min)

Thereafter, glucose increased at around t=90mins, one hour after SRIH was commenced.

Group mean	Start of clamp BG \pm SD mmol/L	Lowest BG concentration mmol/L	Mean BG 0100-0800h	Median coefficient in last 2 hours	Bias of clamp Last 7hours Last 2hours
Group 1 SRIH	9.3 \pm 4.6	4.2 \pm 1.6 at 165mins	5.2 \pm 0.4	4.8% (R 6.9-19%)	+0.27 +0.2
Group 2 SRIH+GH	7.2 \pm 2.2	4.6 \pm 0.9 at 120mins	5.7 \pm 0.4	4.9% (R 2.3-19.6%)	-0.95 -0.59
Group 3 Matthews 1990	9.8 \pm 4.2	4.5 \pm 0.8 at 180mins	5.3 \pm 0.2	4.1% (R 1.4-12%)	+0.26 +0.2

Table 5.4a

The clamp characteristics were compared by group: lowest blood glucose, maximum rate of fall of glucose within the first 3 hours (2000-2300h), the median coefficient of variation of blood glucose in the latter part of clamp (0600-0800h) and the bias of the clamp.

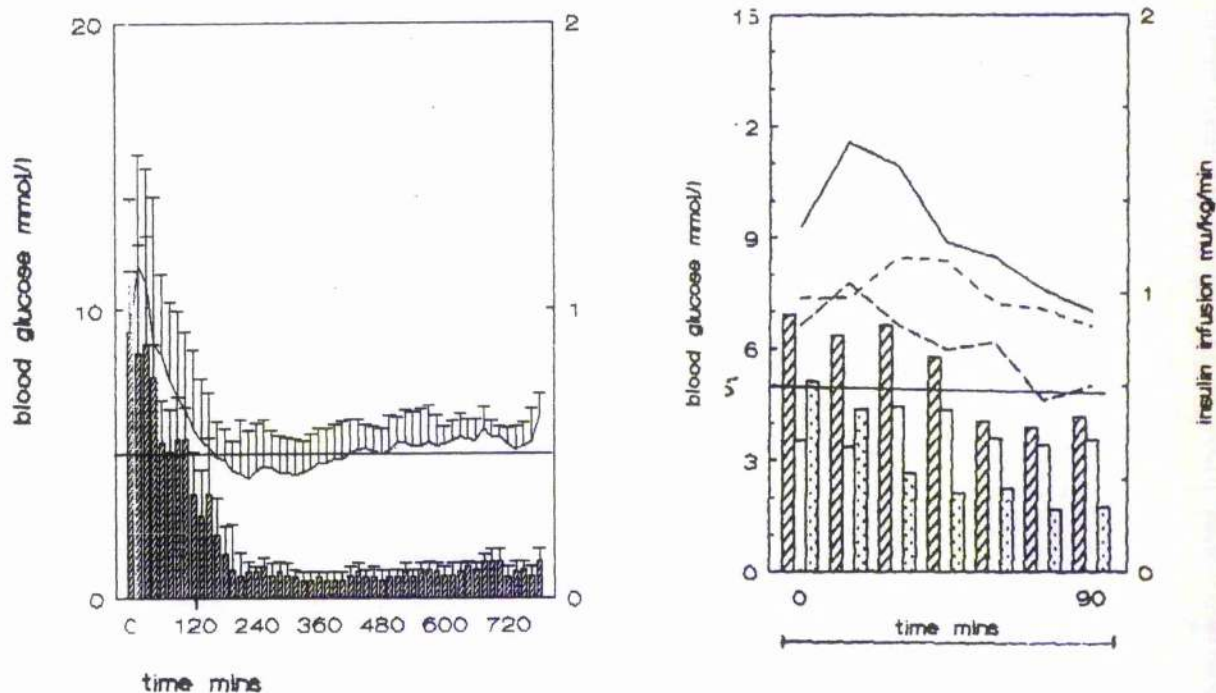


Figure 5b Insulin infusions are steady after 90minutes of SRIH

Left panel shows overnight insulin infusion rate (mU/kg/h) and glucose levels (up-going error bars) for all study nights with SRIF. Note insulin infusion rate is constant after 120mins of staring clamp.

Right panel shows the first 90 minutes of clamp when SRIF action is critical. Dashed lines are glucose levels during study nights.

KEY



Target blood glucose 5mmol/L is represented by the central bold line

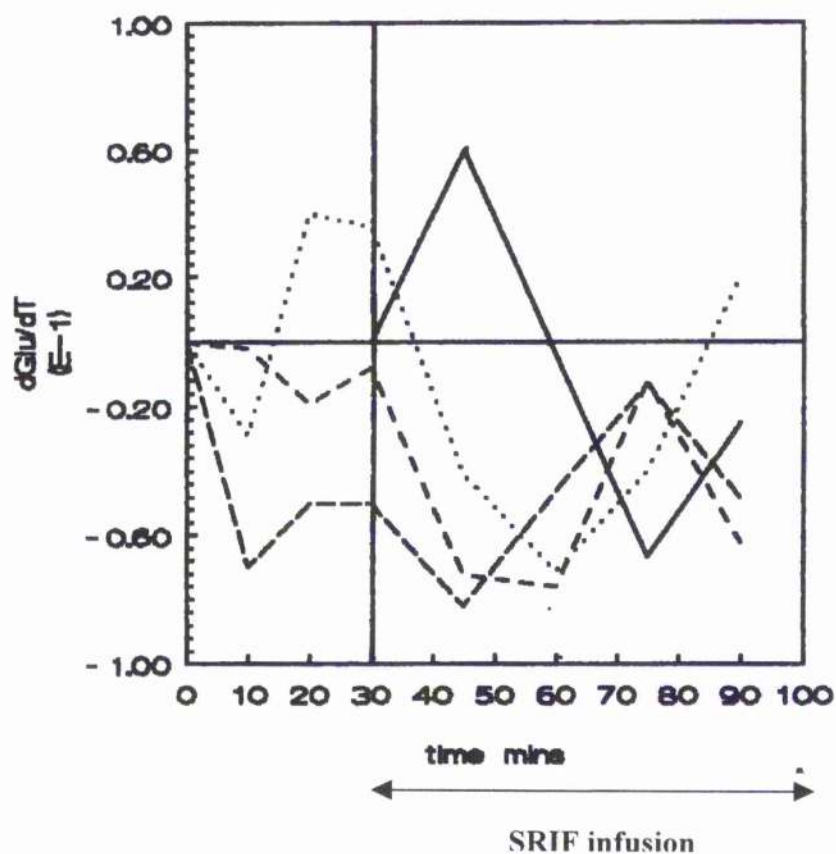


Figure 5c Analysis of rate of glucose declination ($dGlu/dT$) during an SRIF infusion starting at $t=30$ mins.

Solid line represents overnight subjects who were given concomitant glucagon infusion and **dashed lines** represent the 3 clamp conditions during GH clearance studies without glucagon.

Note that although the rate of change in glucose (fall represented by negative values) are similar, the maximum decrement occurs earlier and very suddenly during SRIF alone (0-20minutes) but a fall is seen at 20-40minutes following SRIF when additional glucagon infusion is given.

Conclusions from clamp observations

SRIH readily suppresses glucagon and GH levels and has been used to obtain standard experimental conditions in this Thesis (2.1.8). Severe hypoglycaemia (<2.0 mmol/L) may trigger adrenergic responses and during GH studies may potentially stimulate other metabolites.

When using insulin-varying clamp, the hypoglycaemic effect of insulin was potentiated by SRIH alone as I observed that during the first hour after commencing SRIH infusion, there is an abrupt fall in glucose (dG fall). This can be prevented by glucagon infusion when no significant hypoglycaemia occurred in the presence of glucagon (lowest blood glucose in Group 1, 2 and 3 were similar). The later restitution of blood glucose to normal was not delayed. When compared to previously described data in adolescents with Type 1 diabetes, the nadir of blood glucose was noted to be earlier (before 3 hours) during GH infusion although rate of fall in glucose was similar in all groups (1.8, 1.3 and 1.8 mmol/L/hour in Group 1, 2 and 3 respectively). This was rather surprising particularly as plasma insulin levels were no higher (mean plasma insulin SRIH 55.9 vs. SRIH+GH 55.4 mU/L) and only 3mU/kg of GH had been infused. However, the insulin-like activity of GH does occur within the first 2 hours and perhaps being demonstrated here. The nadir of mean glucose levels was similar in all groups. During the stable part of the clamp (0100-0800h) glucose levels were similar and within clamp limits (mean glucose levels of 5.2, 5.7 and 5.3 mmol/L in Groups 1, 2 and 3 respectively). The clamp stability over the last 7 hours were comparable and within the target range in all groups. However, the bias of the clamp in Group 2 (SRIH+GH) was negative suggesting that as mean glucose values drifted up in the last 2 hours, the difference was lowered.

Very interestingly, mean insulin infusion rates (hence requirements) were far lower during SRIH infusion in clamp period 01.00-08.00h (only 32% during SRIH and 53.1% during SRIH+GH compared to insulin infusion rates used in Matthews, 1990). Mean plasma free insulin levels were 17.2 and 21.8 mU/L

during SRIH alone and SRIH+GH respectively. This confirms that insulin requirements to maintain glucose clamp conditions are much lower during SRIH infusion. This could either be because SRIH potentiates insulin action or decreases insulin clearance (Kollind 1990). Since the dose of SRIH administered was low ($50\text{mcg}/\text{m}^2/\text{h}$), it was not likely that circulatory effects of the peptide alone could explain these divergent actions.

My observations suggest that the interaction of SRIH and insulin is mainly seen during the first hour of SRIH administration, the peptide potentiates the effect of insulin that rectifies by 60minutes. After this time, glucose levels are maintained effectively with good prediction of insulin requirement by the programme used and an appropriate glucose clamp maintained with a constant SRIH infusion. This allows the observation of exogenous GH actions on changing insulin requirements.

5.0.5 GH suppression data

The GH profiles obtained under the different clamp conditions are illustrated in Figures 3.2a.

Using the same dose of SRIH, suppression of endogenous GH was achieved within 60mins (mean \pm sem plasma GH levels at t90: NG clamp 1.6 ± 0.5 ; HG clamp 0.8 ± 0.2 ; HI clamp $2.1 \pm 0.6\text{mU/l}$) when the appropriate glucose/insulin clamp had been established for 90min and again at time t=150min, plasma GH levels: NG clamp 1.9 ± 0.6 ; HG clamp 1.1 ± 0.2 ; HI clamp $3.3 \pm 1.2\text{mU/l}$) (Table 5.4b displays individual subjects' data).

Thus, the plasma GH suppression levels, when using the same dose of SRIH, were noted to be higher during the hyperinsulinaemic than during the hyperglycaemic or normoglycaemic clamps.

Subject	A	B	C	D	E	F	G
NG clamp							
Plasma GH mU/L Mean t60-90	*	3.7 ±1	3.9 ±1	0.5 ±0	3.9 ±0.5	0.5 ±0	1.7 ±0.6
Plasma GH mU/L Mean t120-150	*	1.9 ±0.7	2.8 ±0.6	2.3 ±0.4	3.7 ±0.5	1.3 ±0.9	1.8 ±0.3
HG clamp							
Plasma GH mU/L Mean t60-90	*	3.3 ±1.1	1.5 ±0.3	1.1 ±0.4	1.1 ±0.3	0.8 ±0.2	0.5 ±0
Plasma GH mU/L Mean t120-150	*	0.6 ±0.03	1.1 ±0.2	2.1 ±0.6	2.2 ±0.4	2.2 ±0.6	1.9 ±0.4
HI clamp							
Plasma GH mU/L Mean t60-90	*	2.4 ±0.8	7.4 ±2.0	2.9 ±0.8	9.5 ±3.7	1.4 ±0.3	-
Plasma GH mU/L Mean t120-150	*	5.0 ±1.1	2.2 ±0.3	3.8 ±0.4	4.5 ±0.3	2.1 ±0.2	-

Summary

GH Suppression mU/L	NG N=6	HG N=6	HI N=5	P value HI v HG
GH t60-90	2.4 (0.3)	1.4 (0.3)	4.7 (1.5)	P=0.014
GH t120-150	2.3 (0.4)	1.7 (0.3)	3.5 (0.5)	P=0.008
Mean (SEM)	2.35 (0.3)	1.55 (0.3)	4.1 (1.0)	P=0.002

Table 4b Hyperinsulinaemic clamp antagonises somatostatin suppression of endogenous GH

Clamps: NG=Normoglycaemic HG=Hyperglycaemic and HI=Hyperinsulinaemic GH suppression in both time periods (t60-90 and t120-150) showed significantly higher plasma GH levels during HI clamp>NG>HG.

5.0.6 GH suppression - effect of glucose and insulin

The effect of glucose and insulin on GH suppression by SRIH was examined further by comparing the mean GH suppression over 30min of the two time periods (t60-90 and t120-150) and the relationship to the mean plasma insulin levels and glucose levels achieved over the previous 60minute time period respectively (t30-90 and t90-150) for all clamps (**Figure 5.5d,e,f**). There was no relationship identified between GH suppression and mean blood glucose levels or glucose infusion rates (**Figure 5.5d**).

Mean GH suppression with SRIH during the hyperinsulinaemic clamp over both time periods was significantly different when comparing clamp mornings (viz. t60-90 mean \pm sem GH suppression: **HI clamp** 4.7 ± 1.5 vs. **NG clamp** 2.4 ± 0.5 , $p=0.102$ vs. **HG clamp** 1.4 ± 0.3 mU/l, $p=0.014$; viz. t120-150: **HI clamp** 3.5 ± 0.5 vs. **NG clamp** 2.3 ± 0.4 , $p=0.002$ and **HG clamp** 1.7 ± 0.3 mU/l, $p=0.008$).

When measuring plasma GH at low levels, the mean GH suppression level may be influenced by the limits of the GH assay (sensitivity of assay 0.3 mU/L and intra-assay CV at GH concentration of 2.9mU/L is 8.0%). The confidence interval may allow a better description of reliable suppression limits.

The confidence interval (CI) may be viewed as the range of possible values for the true difference that are statistically likely around the point estimate (mean) values obtained in each clamp i.e. above or below 95% CI would be a probability of seeing a difference of $p < 2 \times 0.025$ (Gardner & Altman 1986).

Further analysis of confidence intervals confirmed a true difference in GH suppression, which was statistically significant:

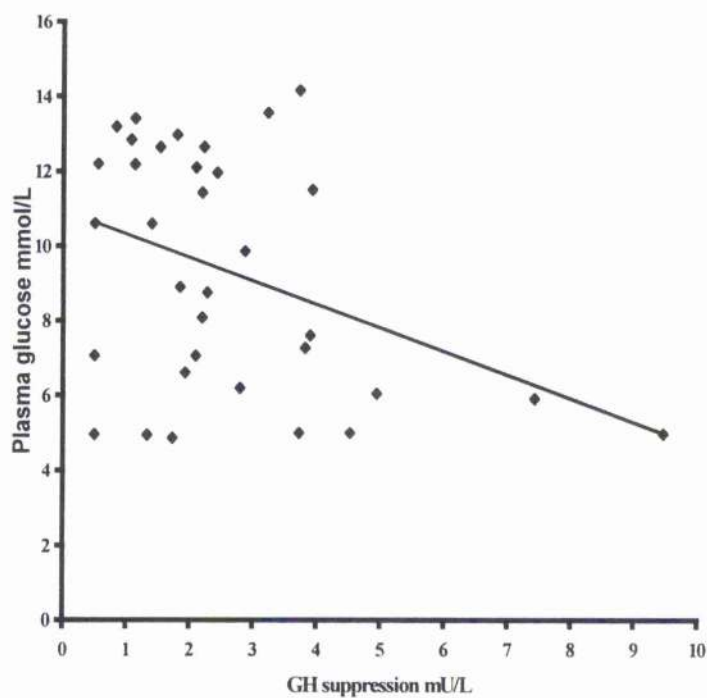


Figure 5d GH suppression: effect of glucose

Individual subjects GH suppression level at t60-90min and t120-150min and plasma glucose levels. There was no significant relationship between glucose levels and GH suppression with somatostatin ($r^2=0.144$, $P=NS$)

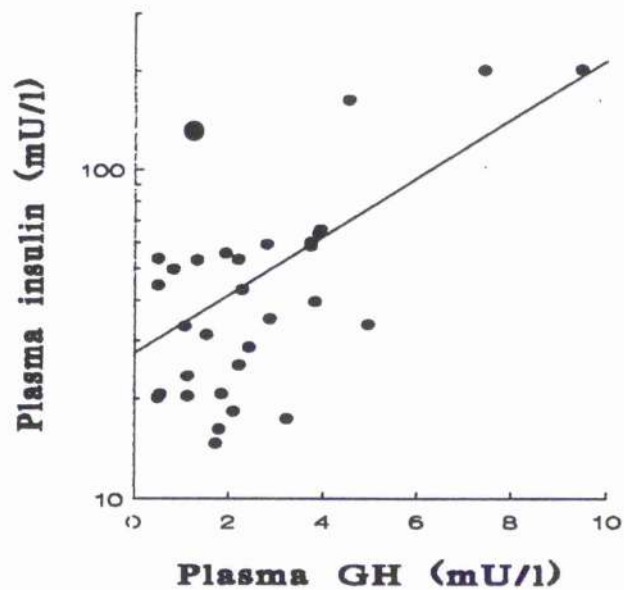


Figure 5e GH suppression: effect of insulin

Individual subjects' GH suppression at t60-90 and t120-150 and relationship to mean plasma insulin (mU/L) (log scale) in respective time periods ($r^2=0.48$, $p=0.004$)

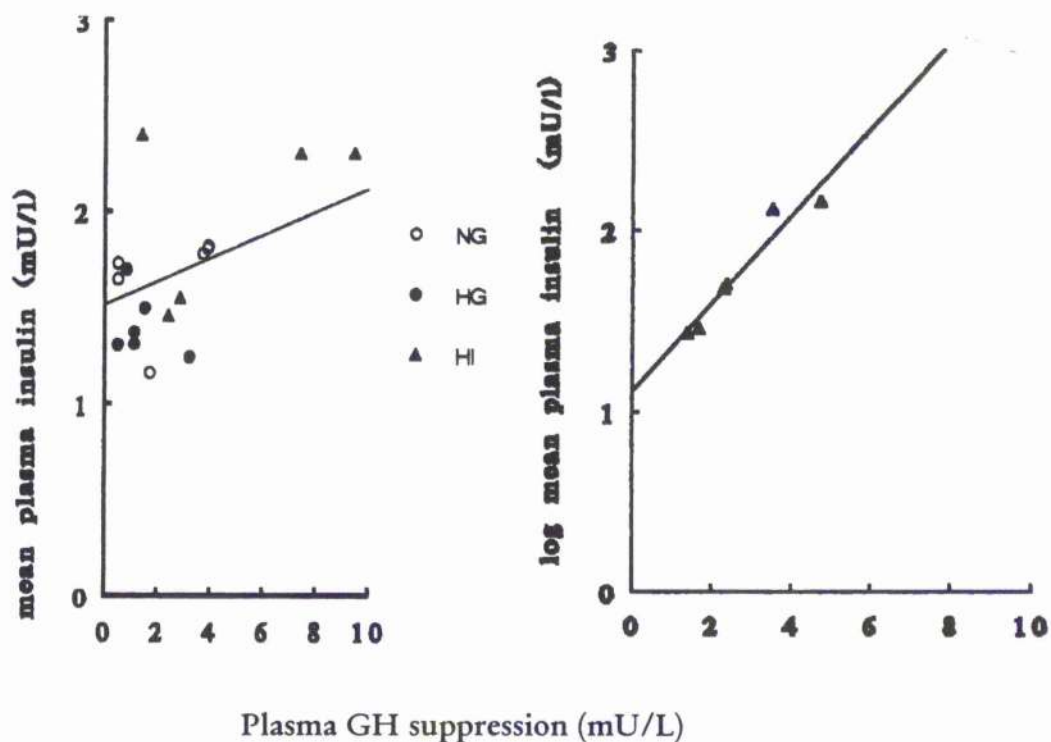


Figure 5f Plasma GH suppression during SRIH is determined by prevailing insulin levels

GH suppression (x axis) during somatostatin infusion and mean plasma insulin (y axis, log scale) has a linear relationship $y = 0.089x + 1.44$

Left panel: Log plasma insulin and plasma GH (suppression) are plotted for subjects' clamp: NG normoglycaemic, HG hyperglycaemic, HI hyperinsulinaemic, Regression line confirms $r=0.6$, $p=0.01$

Right panel: Relationship of mean GH suppression of each clamp (NG,HG,HI) during time periods t60-90 and t120-150 and mean plasma insulin (log scale) in the previous hour. Note linear correlation between GH suppression and plasma insulin ($r=0.96$, $p=0.002$)

Mean GH suppression (95% confidence interval, two-tailed α at $p < 0.05$)

Mean GH suppression 95%CI (mU/L)			
at study time = 90min			
HI clamp	4.7 (0.9, 6.2)	vs.	2.4 (1.1, 3.7) (NG)
			1.4 (0.6, 2.2) (HG)
at study time = 150min			
HI clamp	3.5 (2.2, 4.8)	vs.	2.3 (1.3, 3.3) (NG)
			1.7 (0.9, 2.5) (HG)

Furthermore, mean GH suppression independently during both these time periods showed a correlation ($r=0.96$, $p=0.002$) with the mean plasma insulin level over the previous hour (t_{30-90} and t_{90-150} mins) (Figure 5.5e,f) and was best described best by the relationship

$$\text{Log [plasma insulin]} = A [\text{GH}] + B$$

For individual subjects' data the correlation between mean GH hormone suppression and mean plasma insulin was still significant ($r=0.52$, $p=0.016$) (Figure 5.5f).

5.0.7 GH rebound release- effect of glucose and insulin

The endogenous GH rebound response after stopping SRIH infusion is shown in Figure 3.2a for each clamp.

(i) Responders

Exaggerated GH response (i.e. peak GH > 50 mU/l) was noted in six clamp mornings (4 NG, 1 HG, 1 HI), a moderate GH response (< 50 mU/l) noted in 5 clamp studies (3 HG, 2 HI) and there was no GH response in 6 clamp studies (2 NG, 2 HG, 2 HI) representing all of the study mornings in two subjects (Table 5.4c,d).

Subject	Responders	Non-Responders
No	4 (12 clamp studies)	2 (6 clamp studies)
Median Age years	19.5	21.5
Sex	3 F 1 M	2M
Median weight kg	64.3	76.1
Median height cm	1.67	1.72
Median surface area m ²	1.72	1.89
Median BMI kg/m ²	23.05	25.7
Median HbA _{1c} % (range)	11.8 (8.8 – 15.8)	10.1 (9.7 – 10.4)
Duration Diabetes years (range)	12.6 (6.1 – 20 years)	12 (10 – 14 years)
Total daily insulin Dose (u/kg/day)	42.8 (0.99 u/kg/day)	70 (1.19 u/kg/day)

Table 4c Comparative demographic characteristics of non-responders with subjects who showed endogenous GH rebound after withdrawal of somatostatin suppression.

No clear demographic characteristics could be discerned, but numbers are small.

Subject	B	C	D*	E	F*	G	All clamps Mean (range)	
							Responder	Nonresponder
NG Clamp								
GH at start t=0 mU/L	22.4	43.8	0.5	11.1	0.5	0.8	19.5	0.5
GH peak mU/L	76	120	0.7	151	0.8	50	99.3	0.75
mean GH mU/L	27.9	49.8	0.6	69.1	0.6	19.0	41.5	0.6
HG Clamp								
GH at start t=0 mU/L	4.2	11.8	7.8	3.0	0.8	2.4	5.4	4.3
GH peak mU/L	23.5	40.4	0.9	44.8	1.6	55.6	41.1	1.25
mean GH mU/L	9.4	13.2	0.7	22.2	1.1	18.2	15.8	0.9
HI Clamp								
GH at start t=0 mU/L	6.4	41.6	13.8	7.7	12.9	2.4	14.5	13.4
GH peak mU/L	30.7	15	1.7	104	6.4	48	49.9	4.05
mean GH mU/L	13.2	9.0	1.2	51.5	2.6	-	24.6	1.9
Serum IGF-1 mU/L	NG	0.59	0.68	0.26	0.68	0.36	0.46	
	HG	-	0.66	0.85	0.50	0.72	-	0.736
	HI		0.97	0.34	-	0.50	-	(0.4-.97)
Plasma insulin mU/L	NG	45.87	49.38	37.77	45.05	50.22	16.14	39.1
	HG	18.74	29.95	19.29	25.10	53.43	20.18	23.5
	HI	38.18	169.20	36.61	118.42	180.72	-	108.6

Table 4d Parameters influencing endogenous GH rebound secretion after ceasing somatostatin suppression , are no different between non-responders* and responders.

Note that responders show highest rebound during NG (normoglycaemic) clamp.
Clamps: HG = hyperglycaemic HI= hyperinsulinaemic

There was marked variability of endogenous GH rebound secretion:

1. **Time of GH pulse increase after SRIH termination:** median time 30min, range 10-60min n=11 clamps)
2. **Mean duration** between start of endogenous GH release and GH peak values (median time 30min, range 10-70min, n=11 clamps)
3. **Peak GH:** Peak GH \pm sem levels (n=12 clamps) were highest during NG 99.3 \pm 22.5 (vs. Hyperglycaemic 41.1 \pm 6.7; Hyperinsulinaemic 51.3 \pm 38.9mU/l) but did not reach statistical significance.

However, when examining the differences in clamps for subjects who showed rebound GH secretion, the mean \pm sem plasma GH levels over the rebound period of study (t150-270min) reached statistical significance during NG normoglycaemic (44.7 \pm 9.8 mU/l) vs. their respective HG (16.9 \pm 3.6, Students paired t test, $p < 0.001$) and HI clamp (26.3 \pm 6.8, $p < 0.001$).

(ii) Non-responders

In two subjects there was no GH rise (mean GH levels: NG 0.8 \pm 0.1; HG 1.3 \pm 0.4; HI 4.1 \pm 2.4 mU/l) on all study mornings (n=6). There were no differences between 'responders' and 'non-responders' with respect to HbA1c, duration of diabetes and insulin dose (or other metabolic data) (Table 5.4c).

5.0.8 Metabolites (Table 5.4e)

Although fasting total ketones (β OHB+ACAC) were high on all three study mornings (mean \pm sem: 0.570 \pm 0.148 (NG), 0.758 \pm 0.203 (HG), and 0.738 \pm 0.339 (HI) mmol/L). Ketones were suppressed quickly after commencing glucose/insulin clamp and SRIH infusion. Ketone levels remained constant throughout the study periods (Figure 5.5g) although all subjects had significantly lower levels during NG clamp (mean \pm sem during study period t90-270 β OH: NG 0.029 \pm 0.015 vs. HG 0.043 \pm 0.009, $p < 0.001$; HI 0.048

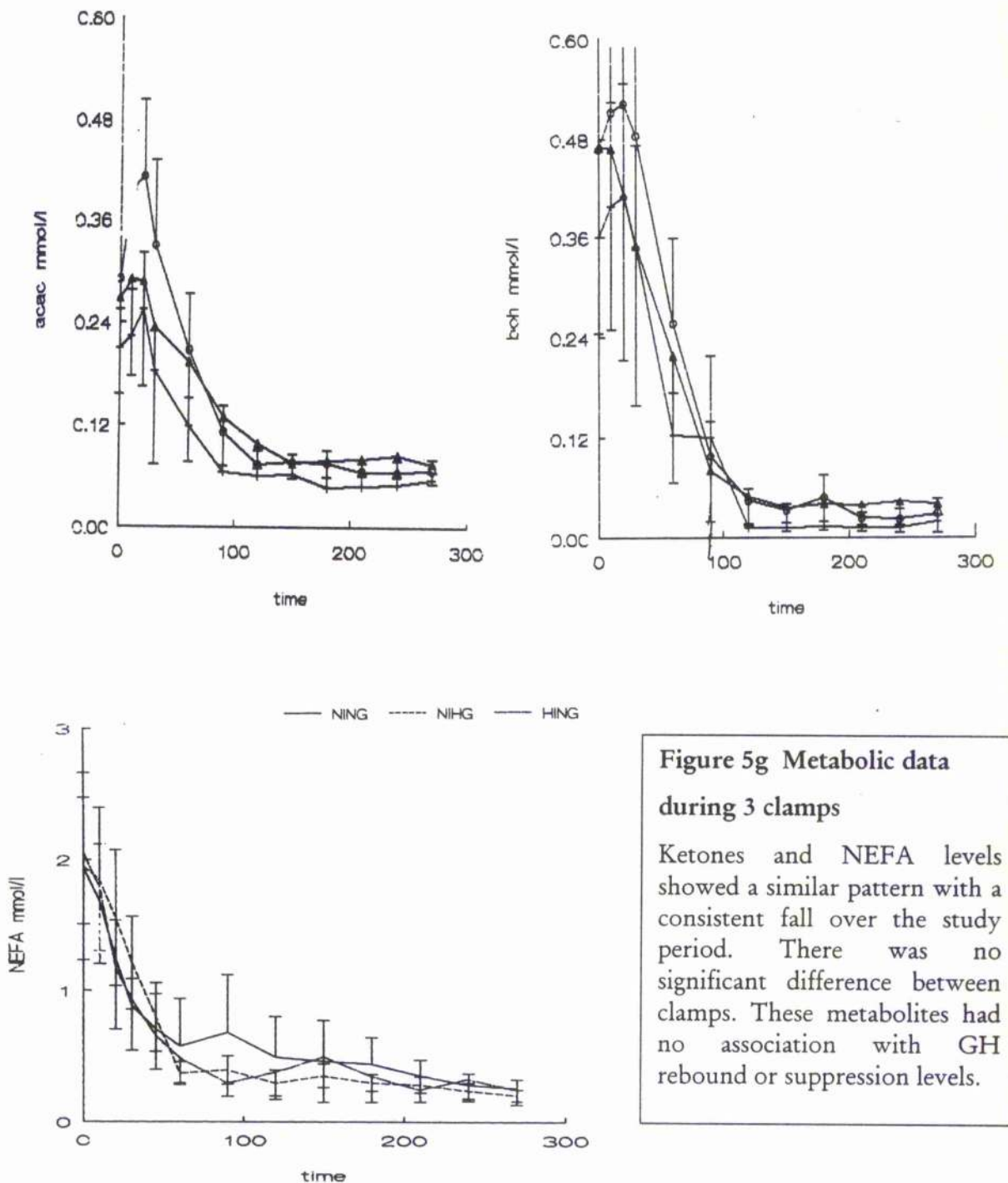


Figure 5g Metabolic data during 3 clamps

Ketones and NEFA levels showed a similar pattern with a consistent fall over the study period. There was no significant difference between clamps. These metabolites had no association with GH rebound or suppression levels.

Metabolic Conditions	Normoglycaemic N=6	Hyperglycaemic N=6	Hyperinsulinaemic N=5
Time 90-150			
Plasma ketones βOHB Acetoacetate Mean (SD) mmol/L	0.121 (0.096) 0.063 (0.002)	0.098 (0.042) 0.087 (0.012)	0.081 (0.059) 0.100 (0.016)
NEFA Mean (SD) mmol/L	0.680 (0.434)	0.395 (0.107)	0.293 (0.099)
Time 150-270			
Plasma ketones βOHB Acetoacetate Mean (SD) mmol/L	0.014 (0.002) 0.053 (0.003)	0.032 (0.005) 0.070 (0.003)	0.040 (0.001) 0.078 (0.002)
NEFA Mean (SD) mmol/L	0.357 (0.041)	0.273 (0.025)	0.332 (0.046)
Time fasting t=0			
Plasma ketones βOHB Acetoacetate Mean (SD) mmol/L	0.359 (0.104) 0.211 (0.045)	0.467 (0.137) 0.291 (0.066)	0.470 (0.225) 0.268 (0.111)
NEFA Mean (SD) mmol/L	1.938 (0.534)	1.948 (0.716)	2.053 (0.547)

Table 4e During morning studies (study time 90-270minutes) metabolic data showed a consistent fall in ketones and NEFA from initial fasting levels.

There was no significant difference noted between clamps as there was a large variation in metabolic data (SD).

Clamps: NG=normoglycaemic HG=hyperglycaemic HI=hyperinsulinaemic

± 0.006 mmol/l, $p < 0.001$ and ACAC: NG 0.056 ± 0.003 vs. HG 0.076 ± 0.006 , $p < 0.001$; HI 0.089 ± 0.008 mmol/l, $p < 0.001$).

Fasting NEFA levels were also high and similar on all study mornings (NG 1.94 ± 0.53 ; HG 2.05 ± 0.54 ; HI 1.95 ± 0.72 mmol/l), fell by 30min after commencing glucose/insulin clamp and were even lower by 90mins. Thereafter, levels remained fairly stable (mean \pm sem NG 0.422 ± 0.055 vs. HG 0.293 ± 0.025 , $p = 0.008$; HI 0.332 ± 0.033 mmol/l, $p = \text{NS}$) but showing a gradual decline over the study period (Figure 5. 5g).

There was no correlation of either ketones or NEFA levels to any of the GH parameters (during suppression or rebound).

5.0.9

SUMMARY AND DISCUSSION

Controversy persists regarding the role of metabolic control in the altered neuroregulation of GH in Type 1 diabetes. Although nutrients such as glucose, free fatty acids and aminoacids have been shown to modulate GH secretion (Imaki 1986), it is not clear from current studies whether these metabolites are stimulatory or inhibitory to GH secretion. As the prevailing metabolic conditions of glucose and insulin may influence the regulation of GH secretion in Type 1 diabetes, I have analysed the effect of different glucose and insulin levels (3 clamp conditions) during somatostatin suppression of GH. After a period of somatostatin suppression of GH, the endogenous GH rebound secretion was also observed during three clamp conditions (NG, HG and HI).

I have clarified that when using the SRIH with insulin, the potentiation of insulin action that commences within 30minutes, has resolved within 60minutes after commencing SRIH. The stability of the clamp is thereafter accurately maintained and experimental observations can be made.

My findings were that trough levels of GH following somatostatin suppression were greater during the hyperinsulinaemic clamp than during either of the other clamp studies. Overall the mean GH suppression during two time periods showed a correlation ($r=0.962$, $p=0.002$) with the mean plasma insulin level over the preceding period. Neither insulin nor glucose affected the endogenous GH rebound (GHRH driven) secretion following somatostatin suppression.

From these observations and the previous chapter, it appears that, in diabetic subjects a reduced somatostatin tone is present because of cholinergic overactivity and this is exacerbated further by excessive insulinaemia which directly or indirectly antagonises somatostatin suppression. Insulin has no effect on the GHRH driven GH secretion.

(i) GH suppression

Jaffe (1993) reports that GHRH, though responsible for the pulse amplitude, it is not involved in the non-pulsatile component or pulse frequency. Interestingly, evidence using antibodies to GHRH and SRIF, suggest that the GH pulse is not related either to hypothalamic GHRH or SRIF but there appears to be other factors causing a direct action on pituitary release.

Some studies in Type 1 diabetes report an altered set point for the inhibitory effects of glucose, insulin and free fatty acids on GHRH-stimulated GH secretion (Schaper 1990) and these changes may be reflecting long term changes in hypothalamic/pituitary function.

The results of my analyses in Chapters 4 and 5 showed that glucose levels did not influence either spontaneous GH secretion or somatostatin suppression. Contradictory evidence regarding the role of glucose exists: normalisation of GH secretion has been demonstrated after a period of improved diabetic control (Tamborlane 1979) but in other studies, GH secretion has remained unchanged (Hermansen 1987) or increased (Edge 1990a). Short-term clamp studies have failed to show any reproducible effects of glucose concentrations on GH secretion. In normal man, hyperglycaemia does suppress GH secretion, presumably by increasing hypothalamic somatostatin secretion (Frohman 1983). This suppressive effect of hyperglycaemia appears to be independent of the concomitant increase in plasma insulin (Schaper 1990).

In vitro and in vivo data from animal studies suggest that in chronic hyperglycaemia, glucose transport into the brain is depressed (Gjedde 1981). GH secretion by 2-deoxy-glucose inducing neuroglycopenia occurs even in the presence of elevated levels of plasma glucose levels, indicating that changes in intracellular glucose metabolism may be more important in determining the effects on GH secretion. Ismail, (1991) also showed that short-term effects of ambient glucose concentration in the range 5-15 mmol/l did not alter GH responsiveness to exercise-induced stimulation. Failure of glucose mediated GH

suppression has also been noted in other studies of subjects with Type 1 diabetes (Press 1992).

My results in this chapter, suggest that somatostatin suppression of endogenous GH is affected by hyperinsulinaemic conditions, either indirectly or directly. The somatostatin dose that I used in my studies was not particularly high (4.8 pmol/kg/min) and yet that led to adequate GH suppression during my studies. Higher infusions up to pharmacological doses of 6mcg/kg/h SRIH have been used to suppress endogenous release (Calabresi 1996).

Cohen, (1991) suggested that there was a threshold infusion rate of somatostatin for significant suppression of GHRH-induced GH secretion and this was higher in subjects with Type 1 diabetes. I am not aware of any comparable data indicating that the somatostatin requirement for the suppression of physiological GH secretion is greater in subjects with Type 1 diabetes. Certainly my data would suggest that a modest dose of somatostatin effectively suppressed GH suppression. A possible contribution of peripheral hypersecretion of SRIH has been hypothesised as a mechanism for downregulation of pituitary SRIH binding in diabetic rodents (Olchovsky 1990). Such an explanation seems unlikely here, as there was no difference in peripheral plasma SRIH administration during all study days.

A direct relationship could be described between GH levels after somatostatin suppression and plasma insulin levels in my studies (Figure 5.5f). Insulin receptors have been identified in the pituitary and the hypothalamus however, experimental data suggests that these cause inhibition rather than stimulation of GH secretion (Yamashita and Melmed 1986). Other studies have demonstrated that hyperinsulinaemia is associated with an exaggerated GH response to GH-releasing hormone (GHRH) in poorly controlled subjects with Type 1 diabetes (Press, 1992) or noted an increase in GH levels during a steady state hyperinsulinaemic euglycaemic clamp (Sharp, 1987). This author also concluded that increased insulin concentrations appeared to have a stimulatory effect on GH release in diabetic subjects with and without retinopathy (Sharp

1984, 1987). In addition as GH increased during a low insulin infusion rate with a further rise during a higher rate, a dose response effect of GH stimulation was seen.

Contrary to this, suppression of GH secretion by insulin has been described by some studies of in-vitro pituitary cells and rats (Melmed 1984, Yamashita & Melmed 1986). Debate thus continues as to whether insulin may have a stimulatory or inhibitory effect on pituitary cells.

Data already suggests that basal and GHRH-stimulated GH release from rat anterior pituitary cells in vitro is not to be influenced by varying glucose or insulin concentrations (Page 1987b). Furthermore, I found that the rebound GH (endogenous GHRH-driven) response following cessation of somatostatin could not be correlated with plasma insulin levels and in fact, the highest rebound responses was seen during the euglycaemic study.

I conclude therefore that the mechanism of increased insulin levels is not stimulatory in influencing GHRH-driven GH release as previously suggested in other reports (Schaper 1990), but insulin appears to act by antagonising somatostatin suppression of GH.

An interesting explanation for insulin action has been proposed by Isaacs and colleagues who have demonstrated that the effect of insulin on rat GH gene expression can either be stimulatory or inhibitory depending on the metabolic state of the cell and therefore insulin may be directly capable of GH regulation in the rat (Isaacs, 1987). Thus high insulin concentrations could produce increased GH levels in Type 1 diabetes.

The somatostatin suppression of GH that is antagonised by increasing insulin exposure, could account for the raised non-pulsatile plasma GH levels seen in diabetes. This may appear to be contradictory in diabetes, where GH levels are increased and the insulin is generally depleted. However, hyperinsulinaemia is a common occurrence when normoglycaemia is attempted (Rizza 1980).

The evidence from in-vitro studies of human pituitary cultures show that insulin can cause inhibition on both GHRH-stimulated and basal GH release in man (Ceda 1985), it has been suggested that these effects may have been mediated by the IGF-1 receptor (Dieguez 1988, Ceda 1987). There are many other ways in which insulin can influence GH and hepatic IGF-1 production.

During hyperinsulinaemia, although glucose utilisation is mainly increased in peripheral tissues, predominantly muscles (DeFronzo 1985) changes in hypothalamic glucose utilisation after intravenous injection of insulin has been described in the rat (Grunstein 1985). Insulin can also affect the hepatic GH receptor number (Baxter 1980b) and post-receptor mechanisms essential for IGF-1 production including mRNA production (Russel-Jones 1992). It appears that although the available evidence indicates that the raised GH levels may result from reduced feedback inhibition from IGF-1, a direct effect of the high peripheral insulin levels in Type 1 diabetes cannot be excluded.

The IGF-1 via receptors on the pituitary cells is known to exert negative feedback on GH release in animal studies (Phillips 1990). Thus, many of the in-vitro effects of insulin may have occurred because of cross-reaction through the IGF-1 receptor. However, it is unlikely that changes in IGF-1 are an important mediator of the inhibition of somatostatin effects on GH suppression that I observed, as short-term administration of high levels of insulin will have no discernible effect on IGF-1 concentration, although would reduce levels of the insulin-dependent IGF binding protein (IGFBP1) (Holly 1988). This IGF binding protein is thought to be inhibitory in most IGF bioassay systems (Taylor 1990) and thus one could predict that a reduction of levels during hyperinsulinaemia would increase rather than reduce IGF bioactivity. Thus, it is most likely that insulin itself, may have regulatory effects on GH release. This may be particularly important when considering the differences in GH regulation in diabetic compared with normal subjects.

(ii) GH rebound secretion

The complete lack of any GH rebound effect after the cessation of the somatostatin infusion in two of the subjects studied was unexpected. I would infer that during a somatostatin infusion, endogenous somatostatin would be suppressed and on withdrawal of the infusion, the GH rebound should reflect GHRH tone and the amount of the GH stored in the pituitary. Somatostatin inhibits the effect of GHRH on GH release (Fukata 1985) but it does not inhibit the stimulation of GH gene transcription caused by GHRH (Barinaga 1985) so that pituitary stores are likely to increase during a continuous somatostatin infusion.

Intravenous administration of GHRH to humans causes a dose-related release of GH from the pituitary although greater inter-individual variation is seen (Evans 1985b). Growth hormone release is detectable within five minutes, maximal at thirty to sixty minutes and returns to baseline by 120 to 180 minutes. Studies have shown no major differences in GH responses to GHRH between male and female responses in normal subjects (Gelato 1986) or a difference between GH responses in neonates, children and young adults. Several groups (Giampietro 1987, Schaper 1992, Guistina 1993) report an exaggerated GH response to growth-hormone releasing hormone (GHRH) in Type 1 diabetes.

I was unable to identify any significant demographic differences between the two subjects who did not show a rebound response following withdrawal of somatostatin and those subjects who did have a rebound response. The mean plasma GH rebound ($t_{150-270}$ min) response in those two subjects who had no rebound response during all 3 clamp studies were similar in all clamp conditions (mean \pm sem GH 1.1 ± 0.1 mU/l, $n=6$). These subjects were not GH deficient as judged by their IGF-1 levels and one subject had a measured GH level of 24.2 mU/l at the start of the clamp study HING. Unfortunately I have no data concerning their GH pulsatility during the previous night and it is possible that they had depleted stores of GH and the study duration of the

study (4.5 hours) was insufficient to replenish these. Alternatively, for some reason, these two subjects may have remained refractory to GHRH stimulation over the period of rebound observation.

The study protocol devised also included an injection of endogenous GH between the two periods of somatostatin suppression. This GH bolus was given in order to determine the half-life of GH under prevailing conditions of glucose and insulin. Schaper (1990) reported impaired auto-feedback by which GH inhibits its own secretion in Type 1 diabetes. I did therefore consider the possibility that the GH bolus may have been affecting subsequent rebound response to GH following somatostatin withdrawal. However, similar peak GH levels were obtained during all of the clamp studies and no differences were seen in the two subjects who failed to subsequently show rebound GH response. Thus I have no reason to believe that short-loop feedback from the bolus could have affected my results.

Consideration that elevated fasting glucose needs to be normalised in most patients before the start of the normoglycaemic (5mmol/l) clamp and that the stimulus to the higher GH rebound could be caused by the falling blood glucose even within the euglycaemic range (DeFronzo 1980). In my study the mean glucose fall (t0-90) was higher during normoglycaemic clamp studies (NG 43%, HI 39.5% vs. HG 7.5%) but although there was corresponding higher mean and peak rebound GH during normoglycaemic clamps, this did not reach statistical significance.

Glucose administration has a biphasic effect on GH secretion in man. The acute inhibitory effect of glucose on plasma GH levels appears to be due to an alteration in hypothalamic activity, since both oral and intravenous glucose inhibit basal and GHRH-stimulated GH release (Johnston 1985, Masuda 1985). The mechanisms involved in the delayed rise in GH have been investigated recently who have shown that oral glucose markedly increases GH responses to GHRH when administered 3.5h prior to GHRH (Valcavi 1990) but cause suppression if glucose is administered 1 hour prior to GHRH (Masuda 1985).

It appears likely that the late rise in GH secretion induced by oral glucose occurs via a non-GHRH-dependent mechanism and possibly as a consequence of reduced release of somatostatin from the hypothalamus. Similar amounts of 10% glucose were administered during the clamp mornings and although, a delay in GH rebound could account for the negative response noted in 2 subjects, it is surprising that during all hyperglycaemic clamps this effect was not seen.

Acetylcholinesterase inhibitors (pyridostigmine) are hypothesised to decrease hypothalamic somatostatin tone. A study by Giustina (1990) showed that in normal subjects pyridostigmine with or without iv GHRH caused a significant increase in GH levels and acted in a synergistic fashion. However in diabetic subjects the GH response to GHRH was variable, some (10/18) showed an exaggerated GH response after GHRH and a lower GH response after pyridostigmine than normal subjects while others (8/18) showed similar response to normal subjects. Duration of diabetes, basal GH levels were higher along with a slightly higher HbA1 in the first group.

In a further study, GHRH stimulated GH production in six diabetic subjects showed that maximal levels were achieved at 15-30 mins and peak GH levels reached were similar ($36 \pm 16.9 \mu\text{g/L}$ ($72 \pm 33.8 \text{ mU/L}$) and $38.7 \pm 18.9 \mu\text{g/L}$ ($77.4 \pm 37.8 \text{ mU/L}$) during hyperglycaemia ($15.3 \pm 0.2 \text{ mmol/L}$) and normoglycaemia ($6.8 \pm 0.1 \text{ mmol/L}$) (Miller 1992) but insulin levels were not reported in this study. In my study, similar peak levels were reached (mean \pm sem GH 99.3 ± 22.5 (NING); 41.1 ± 6.7 (NIHG); 49.9 ± 38.9 (HING); mean all clamps $63.9 \pm 17.9 \text{ mU/L}$, $n=17$) and after a median time of 30min (range 10-70min) after the start of the GH pulse.

Studies in normal man show that short-term elevations of insulin to supraphysiological levels (200 mU/L) while euglycaemia is maintained, do not influence either basal or GHRH-stimulated GH secretion (Schaper 1992). The potentiation of the response to GH-releasing hormone by insulin would indicate loss of an inhibitory effect on the pituitary either directly or mediated

by a reduction in somatostatin levels. My data would support the hypothesis that it would appear that insulin is directly inhibiting the suppressive effects of somatostatin at the level of the pituitary. The mechanisms underlining these observations are likely to be complex.

Short-term hyperglycaemic clamp in my study confirmed that high ambient glucose concentrations had no effect on the ability of somatostatin to suppress the pituitary release of GH.

I did not see increased GH rebound levels (GHRH driven) during hyperinsulinaemic conditions. There is also possibility that hyperinsulinaemia in the studies reported by Press (1992) may be having an effect on endogenous somatostatin production by the hypothalamus. Thus I would conclude from these observations that the effects of insulin on GH secretion are mediated via antagonism of somatostatin effects on the pituitary rather than an effect on GHRH.

In conclusion, this data provides evidence that hyperinsulinaemia, directly or indirectly, may inhibit suppressive effects of somatostatin at the level of the pituitary, whereas it has no effect on the GHRH driven GH response seen following the withdrawal of the somatostatin infusion.

CHAPTER 6

CONVOLUTION AND DIFFERENTIATING THE GH SIGNAL

6.0.0

INTRODUCTION

In this section I report overnight studies in young adults with Type 1 (Insulin-Dependent) Diabetes Mellitus where the effects of continuous or pulsatile GH administration (the GH signal) on ketogenesis, lipolysis, and insulin sensitivity were compared with a control night during which endogenous GH secretion was suppressed with somatostatin. The data following in this section are given for 6 subjects (JLMNP and Q). Nocturnal GH plasma levels found in insulin-dependent diabetes mellitus were reproduced in study subjects. The estimate of GH dose used was calculated from deconvolution estimate and volume of distribution with the aim of mimicking diabetic physiological plasma levels in study subjects. Study design, practical procedures and standard conditions are described fully in Chapter 2 (section 2.1.2-2.1.9).

(i) Cross correlation

Cross-correlation (Chatfield 1975) was used to examine the phase relationships between oscillations in the GH data and insulin requirements. This involves time series data (GH pulses) being correlated with insulin infusion rates, which are then moved to the right of the GH pulses by minute intervals and correlated again. Data in phase have a positive r value at zero lag. If there are regular cycles the serial values of r will fall until they reach a nadir when the data are 180° out of phase. The first positive peak represents the period of any regularly recurring cycle. Data can be pooled using Fisher's Z transformation and the significance can be determined from the r -value. The correlation coefficients are calculated and the phase relationships between oscillations in both data sets can be found by examining the maximum positive correlation.

(ii) Insulin clearance

Metabolic clearance rate for plasma insulin (MRC-I) (mL/kg/min) was determined for the period of euglycaemic clamp during steady state for each study night as a ratio of insulin infusion rate to plasma free insulin (Castillo 1994). Using the formula

$$\text{Metabolic Clearance Rate of Insulin} = \frac{\text{insulin infusion rate} \times 1000}{\text{plasma insulin}}$$

mL/kg/min

(iii) Statistics

Results are given as mean \pm SEM unless otherwise stated. Blood glucose and insulin infusion data were normally distributed. Log-transformation normalised the ketone, lipid and plasma free insulin data and therefore parametric statistical techniques have been used on these log-transformed data. Student's paired t-test was used for comparisons of mean data between profiles and at individual time periods. Analysis of variance (2-way ANOVA) was used to examine changes with time and confirm differences between study nights.

6.0.1

RESULTS

6.0.2 Convolution of GH

(i) GH plasma profile

Comparison of adolescent GH profiles in diabetic and control subjects are illustrated in **Figure 4.3a**. Appendix 6 illustrates further GH plasma profiles seen in diabetic adolescents by puberty stage.

(ii) Mean GH between study nights

GH dose administered ranged between 78-121 mU/h on continuous infusion GH; 312-484 mU/pulse on pulse night and mean GH (20.00-08.00h) levels achieved were similar at 9.7 ± 0.4 and 9.8 ± 1.5 mU/L respectively.

(iii) Separation of GH signal: Pulse versus Continuous Infusion

Characteristics of the GH pulse profile when administered either as a continuously raised baseline (12 hour infusion) or as three pulses are shown in **Figure 6.6a**.

(iv) GH PULSES

During pulse GH administration plasma levels rose by 30min and reached peak plasma levels by 60min after the start of GH infusion. After cessation of GH infusion, the pulse was sustained for 30min (time taken to reach half-peak levels) and had reached suppression levels by 60min. Pulse characteristics achieved in all study patients (n 5) were similar and there was no difference between the first, second and third pulses given. The range for peak-pulse was 26.5-36.9 mU/L. This was within the predicted model requirement.

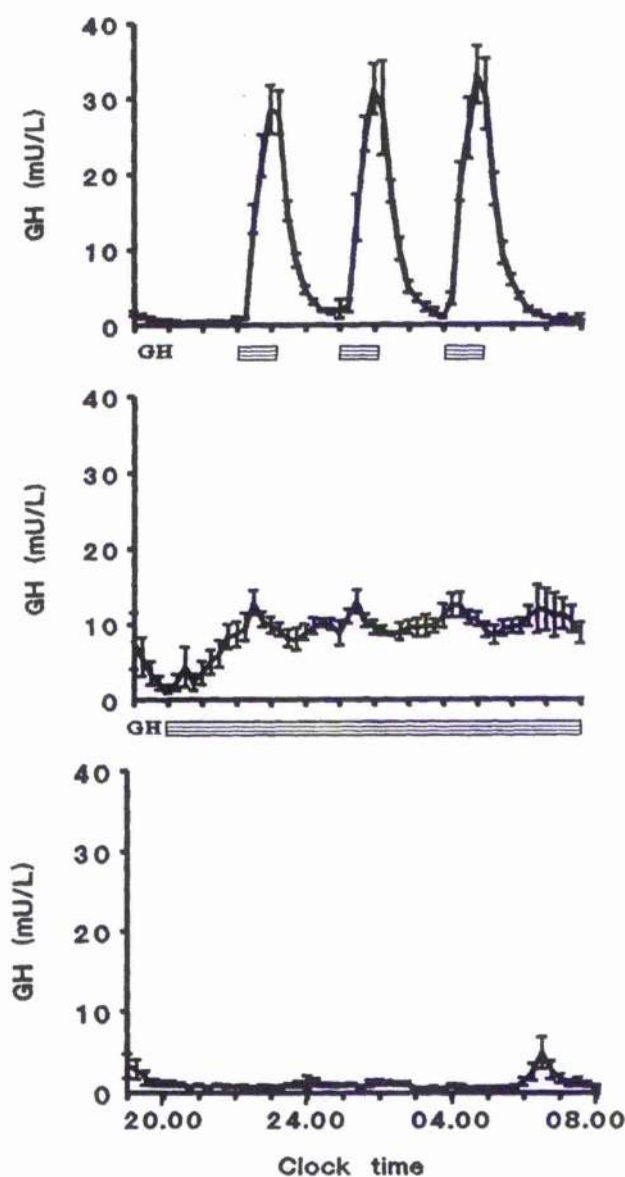


Figure 6a Reconvoluted GH profiles: all subjects

Endogenous GH was suppressed with somatostatin. GH replacement was infused as pulses (6mU/kg/h) (upper panel) or continuous infusion (1.5mU/kg/h) (middle panel). Mean overnight plasma GH levels were similar at 9.7 ± 0.4 and 9.8 ± 1.5 mU/L although the signal differed. **Pulse signal:** There was no difference in characteristics between first, second or third pulse (see text). **Continuous signal:** GH levels maintained between 8.6-10.8 mU/L, although oscillations were noted. ANOVA confirmed no significant drift and linear regression defined a zero slope.

Control night (lower panel) when there was no GH signal: endogenous, GH remained suppressed at mean \pm SEM 1.1 ± 0.1 mU/L throughout the study period 20.00-08.00h

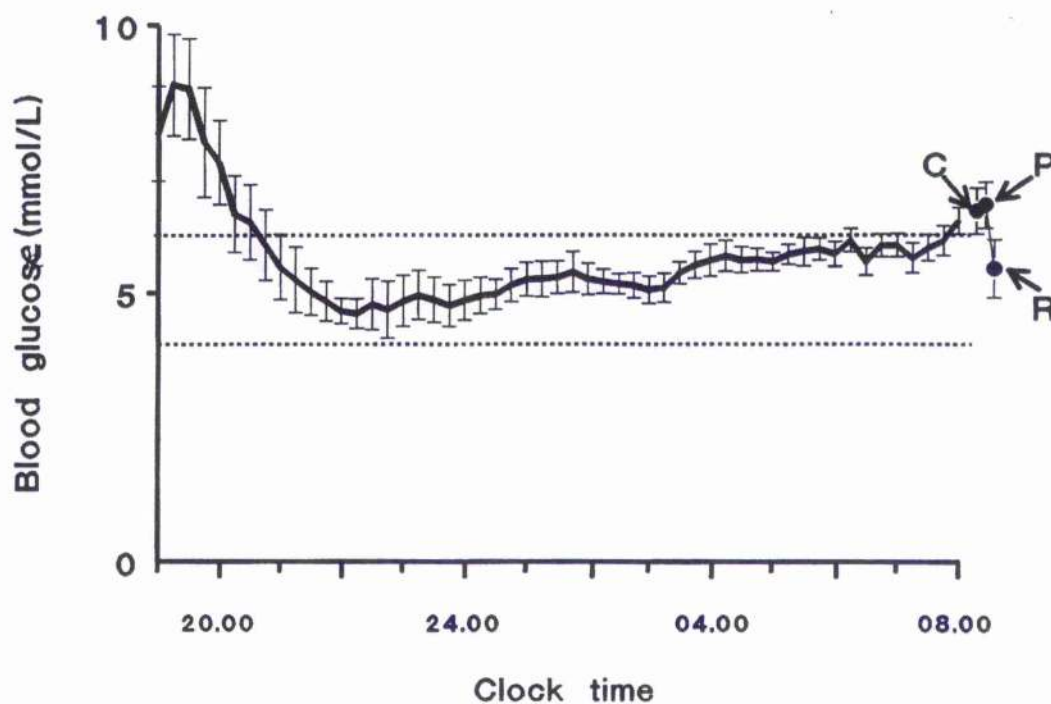


Figure 6b GH signal: glucose clamp

Pooled overnight (20.00-08.00h) blood glucose concentrations (mean \pm SEM) from all subjects during study nights using variable rate insulin clamp. Euglycaemic conditions were achieved (range between dashed lines). Right panel shows the fasting glucose levels were higher on GH nights (P=pulse GH 6.5 ± 0.4 , C=continuous GH 6.4 ± 0.4 , R=control reference (no GH) 5.4 ± 0.5 mmol/L).

6.0.3 Glucose clamp

Comparable normoglycaemic conditions were achieved on all three nights (20.00-08.00h) with mean \pm SEM glucose levels of 5.8 ± 0.1 (pulse GH); 5.5 ± 0.1 (continuous GH); 5.3 ± 0.1 (control) mmol/l.

Early morning blood glucose levels were noted to rise between 05.00-08.00h despite increases in insulin infusion rates during GH study nights (pulse GH 6.2 ± 0.4 vs. control 5.55 ± 0.3 mmol/L, $p=0.001$; continuous GH 5.9 ± 0.3 vs. control, $p=0.002$). This was reflected in a significantly higher fasting glucose levels (pulse GH 6.5 ± 0.4 vs. control 5.4 ± 0.5 mmol/L, $p<0.001$; continuous GH 6.4 ± 0.4 vs. control, $p<0.001$).

6.0.4 Insulin changes

Mean insulin requirement between 01.00-08.00h was highest with pulse GH: 0.17 ± 0.03 mU/kg/min vs. control 0.09 ± 0.01 ($p<0.001$) with only slight increase during continuous GH: 0.10 ± 0.01 vs. control ($p=NS$). This represented a mean increase of insulin infusion requirement of 88.8% during a pulse GH night (Table 6.5a).

Plasma free insulin levels (01.00-08.00h) were correspondingly higher on the pulse GH night: 26.53 ± 3.01 mU/L vs. control 17.16 ± 0.42 ($p=0.01$); pulse vs. continuous GH 17.88 ± 1.55 ($p=0.01$). During a pulse GH night despite a 88% increase in insulin infused, mean blood glucose was higher by 12.7% in the same time period compared to control night.

(i) Insulin sensitivity and insulin clearance and GH

Plasma free insulin concentrations during steady state euglycaemia were used as an index of insulin sensitivity. This suggests that only pulses of growth hormone mediate the decrease in insulin sensitivity (mean rise of insulin requirement by 88.8% compared to control and continuous GH infusion).

Time Period	Insulin requirement mU/kg/min			Insulin Clearance ml/kg/min		
	Pulse	Continuous	Control	Pulse	Continuous	Control
*22.00-01.00h	0.122 ± 0.054	0.143 ± 0.040	0.087 ± 0.024	-	-	-
01.00-04.00h	0.1971 ± 0.092	0.111 ± 0.022	0.070 ± 0.009	7.1 ± 2.1	4.8 ± 1.1	4.2 ± 0.1
04.00-08.00h	0.157 ± 0.069	0.119 ± 0.015	0.102 ± 0.022	6.2 ± 2.1	7.4 ± 0.5	5.8 ± 0.4
mean *01.00-08.00h	0.173 ± 0.081	0.115 ± 0.022	0.086 ± 0.022	6.5 ± 1.5	5.6 ± 0.8	5.1 ± 0.2
% insulin change*	29.5%	- 24.3%	-1.2%			

Table 5a Insulin requirements increase only on pulse GH night without significant alteration in insulin clearance.

The Metabolic Clearance rate of insulin (MCR-I) was calculated from the insulin infusion rate divided by the plasma insulin.

These data suggest that alteration of insulin sensitivity occurs on pulse GH night.

Study night	Insulin infusion rate mean (SEM) mU/kg/min	Plasma Insulin mean(SD) mU/L	Glucose mean (SEM) mmol/L
Clock time	01-08h	01-08h	01-08h
Pulse			
J	0.113	7.72	7.08
L	0.113	31.95	6.06
M	0.285	28.97	5.52
N	0.228	52.93	5.49
P	0.139	11.11	5.35
Q	0.157	11.71	4.92
Mean	0.173 +0.081	24.10 +12.7	5.74 +0.3
Continuous			
J	0.1247	11.7	5.19
L	0.0983	37.15	5.59
M	0.1271	17.78	5.92
N	-	-	-
P	0.0752	9.79	5.29
Q	0.2129	13.14	5.31
Mean	0.115 +0.022	17.9 +6.7	5.46 +0.2
Control			
J	0.0214	8.62	5.01
L	0.0654	22.49	5.30
M	0.0847	14.39	4.98
N	0.1235	39.09	5.01
P	0.1656	10.83	5.35
Q	0.0570	7.59	5.12
Mean	0.086 +0.022	17.16 +7.2	5.13 +0.1

Table 5b GH pulse signal compared to control, significantly increases insulin requirements ($p < 0.001$) and plasma insulin levels ($p = 0.002$) during overnight euglycaemic clamp.

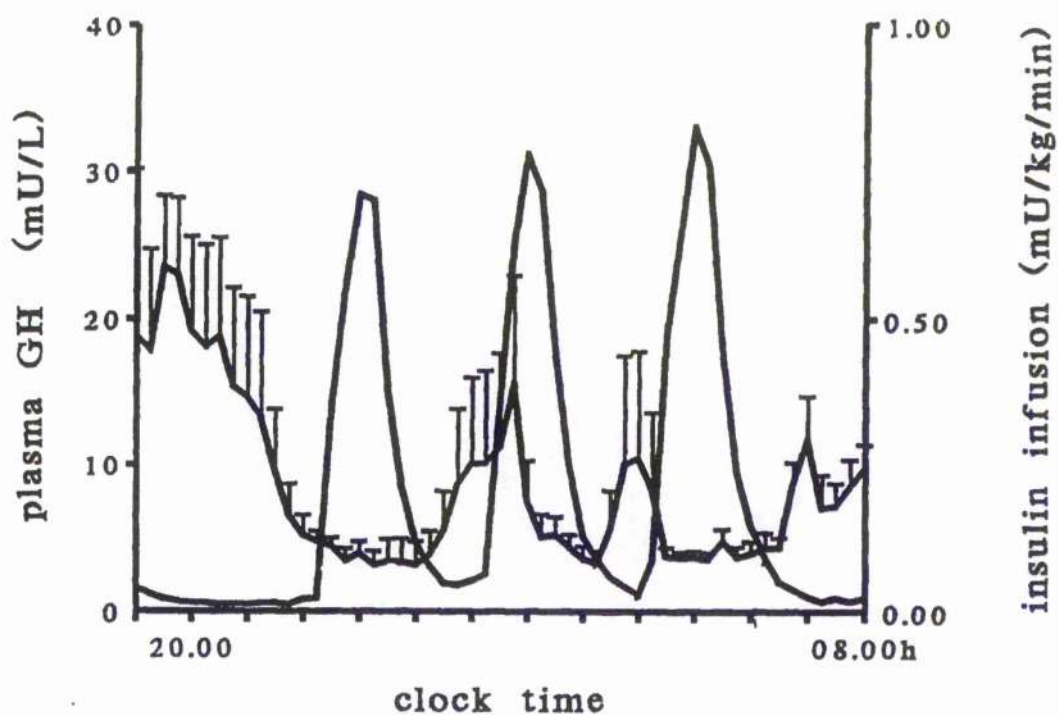


Figure 6c GH signal: insulin requirements (infusion rate)

The overnight oscillations of insulin infusion requirements (solid line and error bars) and the plasma concentration of GH (solid line) indicate a oscillating pattern.

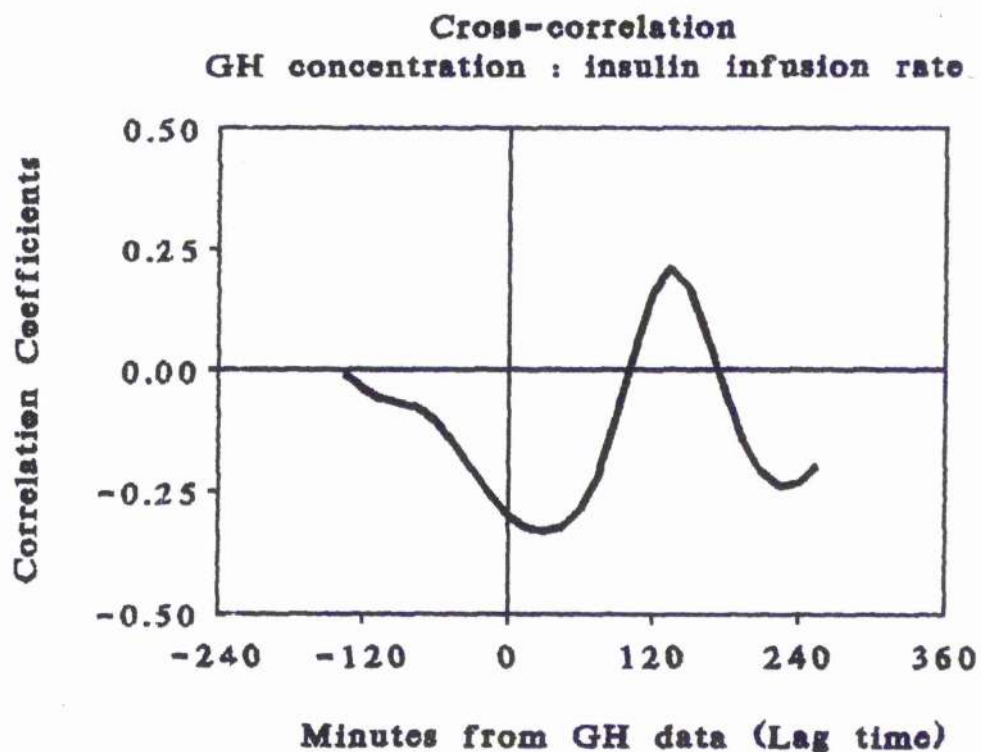


Figure 6d **GH signal: Cross-correlation**

The relationship between GH concentration and insulin infusion rate shown here as a correlogram. A maximum significant positive association is seen at 135minute lag time ($r=0.21$, $p<0.001$), indicating that on average maximum insulin infusion rates were required 135minutes after a GH pulse.

When examining overnight insulin clearance during time periods 2000-2400, 0100-0500 and 0500-0800h, the mean clearance was similar (Table 6.5a). Insulin sensitivity and clearance were not related to either individual peak GH levels nor mean plasma GH ($p=NS$). Thus, overnight (01-0800h) insulin clearance showed no significant difference between study nights. To establish whether there was a dawn rise in insulin requirement consecutive 4 hour time periods were compared between 23.00 and 0.800h. A rise in insulin requirement was observed only when comparing the time period 23.00h-01.00h versus mean overnight insulin requirements (01.00-08.00h) (Table 6.5a and 6.5b).

(ii) Insulin requirement and GH pulsatility

During the pulse GH night, there was a visible oscillatory change in insulin requirements (Figure 6.6c). There appeared to be a rise in insulin requirement with a phase lag of approximately 2 hours following a GH pulse, which was maximal at 3 hours and had fallen again by 4 hours. Cross-correlation was used to confirm the time-relationship between GH and insulin oscillations.

(iii) Relationship between GH oscillations and Insulin

Cross-correlation between insulin infusion rates and GH pulses showed a maximal significant positive association ($r = 0.21$, $p < 0.001$) at 135min (Figure 6.6d) indicating that the insulin infusion data are displaying a time-related oscillatory pattern where an increase in insulin requirement lags 135min after GH pulse concentration.

6.0.5 Metabolite changes

Metabolic data were analysed after obtaining stable clamp conditions during the period 01.00-08.00h. The pattern of β -OHbutyrate and acetoacetate concentrations is shown in Figure 6.6e. After an initial fall from 19.00h levels, plasma ketones rose steadily from approximately 21.00h (continuous GH night) and 23.00h (pulse GH night) until peak levels were reached between 01.00-

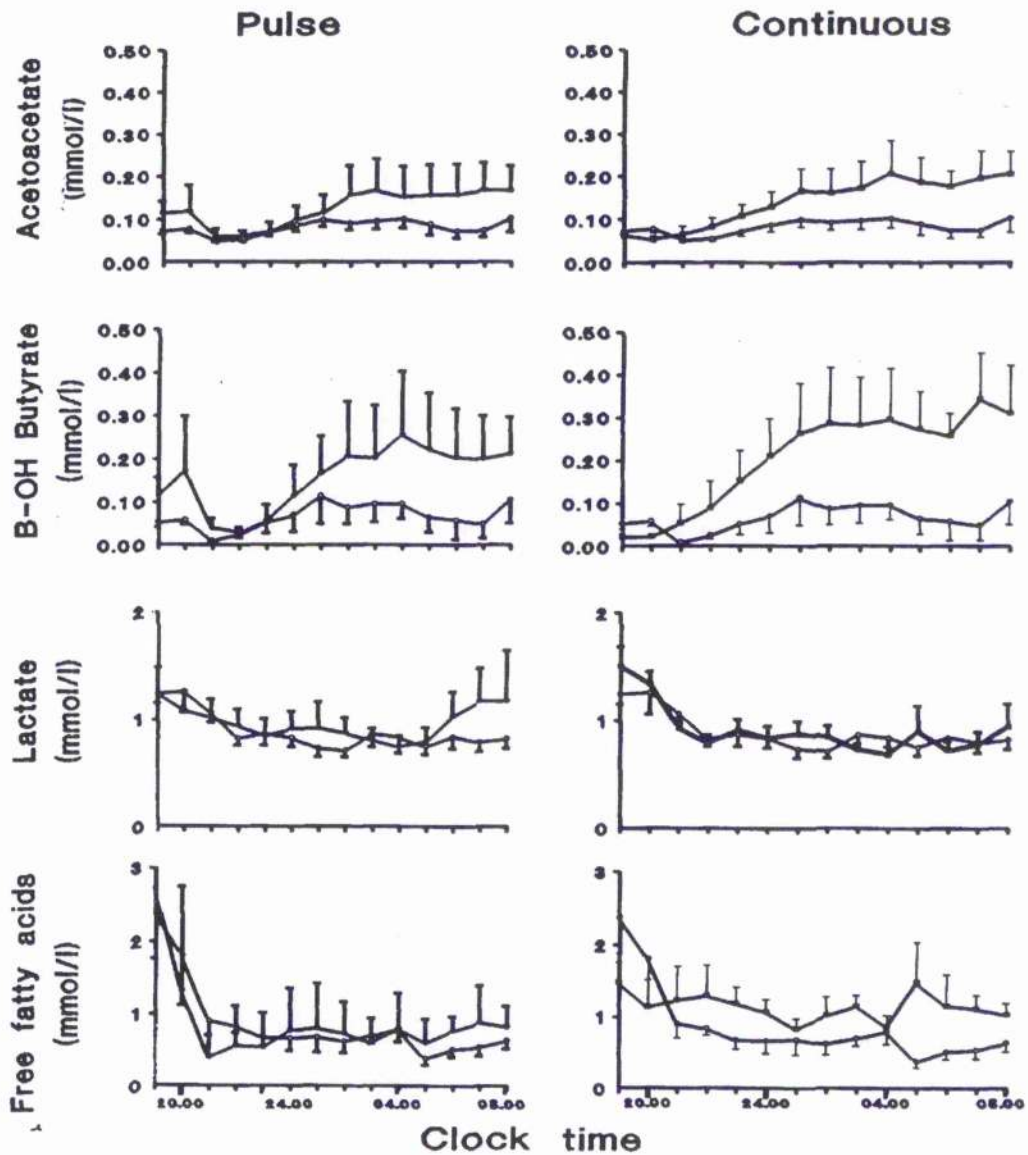


Figure 6.6e GH signal: Metabolic data

Results of mean overnight metabolic data (ketones, lactate and NEFA). Left panel shows the results from pulse GH signal; the right panel shows results after continuous GH administration.

Up-going error bars (SD) on data from GH night and down-going from control night.

Abscissa = clock time, Ordinate concentrations of acetoacetate, β OH butyrate, lactate and free fatty acids respectively.

Study night	β OHButyrate Mean (SEM) mmol/L			Acetoacetate Mean (SEM) mmol/L			Lactate Mean (SEM) mmol/L		
Clock time h	01-08	SEM		01-08	SEM		01-08	SEM	
Pulse									
J	0.365	0.031		0.284	0.082		0.711	0.024	
L	0.357	0.042		0.263	0.023		0.616	0.050	
M	0.038	0.009		0.046	0.004		1.149	0.182	
N	0.011	0.002		0.041	0.002		1.617	0.148	
P	0.190	0.041		0.065	0.013		0.670	0.035	
Q	0.295	0.078		0.240	0.025		0.874	0.082	
Mean (SEM)	0.209	0.014		0.156	0.010		0.939	0.035	
Continuous									
J	0.468	0.048		0.290	0.032		0.651	0.030	
L	0.130	0.031		0.118	0.016		0.955	0.054	
M	0.245	0.028		0.129	0.010		0.664	0.055	
N	-	-		-			-		
P	0.082	0.004		0.056	0.006		1.282	0.127	
Q	0.528	0.050		0.331	0.038		1.221	0.156	
Mean (SEM)	0.289	0.014		0.185	0.017		0.955	0.038	
Control Reference									
J	0.241	0.043		0.148	0.055		0.608	0.020	
L	0.051	0.013		0.073	0.009		0.670	0.059	
M	0.034	0.009		0.063	0.012		0.750	0.048	
N	0.007	0.001		0.047	0.002		0.916	0.085	
P	0.012	0.004		0.066	0.008		1.018	0.092	
Q	0.138	0.054		0.148	0.061		0.706	0.055	
Mean (SEM)	0.084	0.008		0.091	0.010		0.778	0.024	

Table 6.5c Mean overnight metabolites are increased by both a continuous and pulse GH signal

02.00h. The level of ketones was sustained at this high level until 08.00h. The rise in 3-OHbutyrate was most exaggerated during continuous GH infusion night and was different to control night at all time periods. These changes reached significance between 05.00h and 08.00h resulting in high fasting levels. Mean (SEM) overnight (01.00-08.00h) 3 β -OHbutyrate levels (**Table 6.5c**) were significantly increased on both GH nights (continuous GH 0.2898 ± 0.014 vs. control 0.0840 ± 0.008 mmol/L, $p < 0.001$; pulse GH 0.209 ± 0.014 vs. control, $p < 0.001$) and fasting (08.00h) levels were increased: continuous GH 0.3116 ± 0.090 vs. control 0.1057 ± 0.055 mmol/L, $p < 0.05$; pulse GH 0.2130 ± 0.084 vs. control, $p = \text{NS}$.

Plasma acetoacetate concentrations followed a similar pattern with significant but less pronounced changes during GH administration (**Table 6.5c**). (Mean (SEM) 01.00-08.00h: Both continuous GH (0.1848 ± 0.017) and pulse GH (0.1562 ± 0.010) were significantly greater than control (0.0908 ± 0.010 mmol/L, $p < 0.001$). Fasting: continuous GH 0.206 ± 0.053 vs. control 0.1042 ± 0.034 mmol/L ($p < 0.05$), pulse GH 0.1678 ± 0.057 vs. control ($p < 0.02^*$).

Two-way Anova on log-transformed data confirmed that ketone levels changed with time between (20.00-08.00h) on all study nights ($F 5.95, p < 0.001$) but had reached plateau levels between 01-08.00h ($F 0.7862, p = \text{NS}$) and that the mean ketone levels were significantly different between the three study nights ($F 83.56, p < 0.001$) being most pronounced between continuous GH and control ($F 111.73, p < 0.001$). High variation of ketone data was noted between subjects on pulse GH nights (**Figure 6.6e**).

The mean fasting *total ketone* levels were elevated Control 0.2099; Continuous GH 0.5176; Pulse GH 0.3808 mmol/L

Lactate levels fell initially (from 20.00h level) and did not vary over time during the later period (01-08.00h $F 1.13, p = \text{NS}$) but there was a small but significant difference between both pulse GH ($F 5.9, p < 0.05$) and continuous GH ($F 3.87, p < 0.05$) and control night detected by ANOVA. Mean plasma lactate levels were only slightly raised with GH administration: Mean level (01-08.00h) on

continuous GH 0.955 ± 0.038 and pulse GH night 0.939 ± 0.035 vs. control 0.778 ± 0.024 mmol/L ($p=0.045$) (Figure 6.6e).

6.0.6 NEFA changes

Overnight (01.00-08.00h) mean levels of free fatty acids (NEFA) were increased slightly and reached significance during continuous GH 1.09 ± 0.07 versus 0.61 ± 0.05 mmol/L control ($p=0.001$); but were less marked during pulse GH 0.94 ± 0.04 versus control ($p=0.042$). Fasting levels however were not significantly different: continuous GH 1.04 ± 0.16 , pulse GH 0.83 ± 0.13 , control 0.64 ± 0.13 mmol/L) as there was a large variation between subjects. Anova confirmed that NEFA levels did not change with time between 01.00-08.00h (F 0.50) but there was a definite difference in mean plasma levels which were higher during continuous GH administration compared with both control (F 27.08, $p < 0.005$) and pulse GH (F 19.15, $p < 0.005$) over this time period. (Figure 6.6e).

6.0.7 Other hormones

2 patients had hourly cortisol and 4 patients had 2 hourly IGF-1 levels measured (details provided in Appendix 6). Glucagon levels were not assayed as previous work has shown adequate plasma concentrations at the infusion rate given of 1ng/kg/min. Cortisol levels showed a similar profile between nights in the 2 subjects with a morning rise. Levels of IGF-1 measured showed no significant change overnight¹. No significant difference in the mean or 08.00h levels of IGF-1 measured by radioimmunoassay were observed when the three study nights were compared although, pulse GH nights appeared to cause a rise in IGF-1 in keeping with other reports.

¹However, I did not however carry out measurements of IGF binding proteins, as BP3 binding protein is thought to be inhibitory in most IGF bioassay systems (Taylor 1990) and IGFBP-1 responds acutely to insulin levels.

6.0.8

SUMMARY AND DISCUSSION

Six young adults with Type 1 diabetes were studied on three occasions overnight. On each night somatostatin and glucagon were infused continuously and r-hGH (total dose 18mU/kg) was given in phosphate buffer as either: 3 discrete hourly pulses (6mU/kg/h, 120 mins apart) or a 12h infusion (1.5mU/kg/h) or buffer solution only as control night. The study thus achieved peak pulse level which were 3.35 times the continuous GH and reflected the similar alteration seen in diabetic subjects (section 6.0.2 and 4.0.7, page 103). The pulse signal causing these metabolic effects was thus differentiated and contrasting metabolic effects were seen.

(i) Differentiating the GH signal

GH is known to have both insulin-like and insulin-antagonistic properties (Davidson 1987). *In vitro*, insulin-like activity occurs soon after the tissue is exposed to GH and includes increased glucose utilisation and related pathways and anti-lipolysis. There is controversy surrounding an insulin-like effect of GH *in intact* animals and humans as several investigators have been unable to demonstrate one (Batchelor 1976, Metcalfe 1981). The lack of the hypoglycaemic effect *in vivo* has been ascribed to the simultaneous rise of portal vein insulin and glucagon, which neutralise each other (Okuda 2001). However, other researchers have found a transient hypoglycaemic effect of GH that was mild and transient lasting only approximately one hour (Goodman 1981). *In vitro* studies have also assessed the role of GH on insulin action. Tissue effects of GH are much more easily seen if the tissue has not been previously exposed to GH (Hart 1984). In liver glucose conversion to glycogen was increased transiently by GH during the first hour of incubation but not after 1.5 hours (Honeyman 1980). Furthermore, exposure of the tissue to GH

only during the first fifteen minutes of incubation rendered it refractory to the hormone during the fourth hour (Goodman 1981). In spite of the insulin-like effects of GH demonstrated under various experimental conditions, it is doubtful whether these play any physiological role. I did not observe any insulin-like effects of GH during overnight GH infusions when using an insulin-varying clamp.

Exposure of tissue to GH *in vitro* certainly causes insulin-antagonistic effects. Interestingly, when human fibroblasts were cultured with extremely high concentrations of GH, glucose uptake was suppressed during the first four hours of incubation but returned to normal by 20 hours (Takeda 1984). In normal subjects, Sheu (1990) determined that hepatic glucose production could be regulated (suppressed to approximately 50% of the original value) by varying the rate of exogenous glucose infusion during glucose clamp studies in the absence or presence of glucagon (5.2 pmol/m²/min) during SRIH infusion. Orskov (1989) showed that physiological increments of circulating GH (30 mU/L) during both 6 and 14 mmol/L glucose clamp impaired glucose-induced glucose uptake and increased endogenous glucose production. This effect was more pronounced during somatostatin infusion (large doses of 250 and 750 micrograms per hour) without GH (44% glucose uptake with GH versus 97% without GH). Increased glucose output and hyperglycaemia still occurred with hypoinsulinaemia and normoglucagonaemia. The same plasma levels increased glucose production 2-3 fold during insulin deficiency.

The mechanism for this effect is still unclear. Reductions in insulin receptor numbers at the tissues could affect insulin action: there is evidence that factors causing abnormalities in the post-receptor mechanisms could cause both reduced insulin sensitivity and reductions in insulin receptor numbers and hence insulin mediated clearance (Unger 1998); GH could be acting directly through its own receptor interfering with insulin signalling at a post receptor level (Muller 2001). GH could also act indirectly through mobilisation of NEFAs from adipose tissue (Hettiarachchi 1996).

(ii) The Dawn Phenomenon

The term "dawn phenomenon" has been limited to indicate specifically the increase in insulin requirements (or development of hyperglycaemia if the need for insulin is not met) in the absence of declining insulin delivery, as well as in the absence of nocturnal hypoglycaemia (Bolli 1988). Such a definition implies that the dawn phenomenon can only be accurately assessed as the cause of fasting hyperglycaemia in diabetic subjects in whom insulin deficiency is prevented. During previous studies, the plasma insulin concentrations usually fell overnight after the evening injection of depot insulin (Francis 1983). Many of the earlier studies of the dawn phenomenon in adults thus involved technical problems with loss of available insulin and waning of insulin delivery (Brennan 1985, Harris 1986, Campbell 1986).

The practical contribution of insulin deficiency to the dawn phenomenon could not easily be separated during such clinical observations. In addition, the heterogeneity of the study population, varying study conditions used and the different definitions applied to the dawn phenomenon added further confusion. Many reports suggest that virtually all (80-100%) Type 1 diabetic patients exhibit a dawn phenomenon (Perriello 1991, Perriello 1988, Campbell 1986, De Feo 1986, Edge 1990b, Beaufrere 1988). By measuring the 2400-0700 increase in plasma glucose concentrations during overnight insulin delivery at a fixed rate, the initial Biostater studies show that the dawn phenomenon accounted for quite a large increase in plasma glucose concentrations i.e. 5-7mmol/l (Campbell 1985b). However, in studies in which the artefact of insulin waning was prevented, plasma glucose concentrations increased by only 1.5-2mol/l in the period from 2400-0700 (Perriello 1988, 1990). Thus it is likely that the specific contribution of the dawn phenomenon to fasting hyperglycaemia is quite modest. Attea (1992) suggests that even an increase of 1 mmol/l or more of plasma glucose was clinically significant during the early morning. Results from this chapter show that even when using a variable insulin clamp and

providing optimum insulin with higher insulin rates during pulse GH nights, fasting glucose concentrations were higher than control on both GH nights (pulse GH 6.5 ± 0.4 versus control 5.4 ± 0.5 mmol/L, $p < 0.001$; continuous GH 6.4 ± 0.4 versus control, $p < 0.001$). Thus, a *fasting glucose* level does not adequately reflect the alterations in overnight insulin resistance or actual insulin requirement.

However, the extent to which the dawn phenomenon contributes to early morning glycaemia remains controversial, partly because comparison of overnight time-periods differs between studies. Insulin sensitivity has been found to be greater in the 2400-0200 period, compared with the 0600-0800 period, both at the level of the liver, as well as the peripheral, insulin-dependent tissues in conditions of euglycaemia (Perriello 1990) contrasting the observations of Widmer, (1988) who found no apparent change in insulin sensitivity between the 0100-0200 and 0700-0800 periods in a group of Type 1 diabetic patients.

In my studies when comparing mean insulin requirements during time periods 01-04.00h and 05-08.00h, there was no consistent mean or individual change between study nights (Table 6.5a). However, when comparing mean insulin requirement change between 22-01.00h and 01-08.00h, a clear incremental change in insulin requirements occurred on a pulse GH night (29.5% increase vs. control -1.2%).

Various non-hormonal and hormonal factors have been implicated for the decreased insulin sensitivity at dawn period (05-0800h) compared with the early night hours but most reports now focus on the parameters of the GH pulse. It has been suggested that reductions in insulin sensitivity at dawn are attributable to the effects of GH on peripheral glucose metabolism (Amiel 1991). Prolonged hyperglycaemia appears to produce regular 100-min plasma insulin oscillations that entrains cyclic changes in peripheral glucose uptake, while hepatic glucose efflux was inhibited (Bowden 1980). More recent studies

however show that presence of insulin secretion rhythmicity is *independent* of glucose variations (Bizot-Espiard 1998).

My results suggest that the nocturnal insulin requirements are triggered only by GH pulse signal. Although the total dose of r-hGH delivered was identical, continuous GH had no significant effect on changing insulin requirements (Table 6.5a) nor were free insulin levels any higher from control night during euglycaemic clamp (mean(SD) insulin 01-08h: continuous 17.9(6.7) mU/L vs. 17.16(7.2) control, Table 6.5b). This suggests that either raised GH pulse amplitude and/or GH pulsatility (pulse frequency) causes insulin resistance.

Perriello in a study of subjects with Type 1 insulin-dependent diabetes mellitus came to similar conclusions although they compared the effects of basal versus basal and pulse GH administration (Perriello 1990). Removal of GH pulses resulted in complete abolition of the increased rate of hepatic glucose production at dawn. Replacement of nocturnal spikes of GH faithfully reproduced the increase in hepatic glucose production at dawn, supporting my findings of an increased insulin requirement to maintain euglycaemia occurring only during a GH pulse night.

My observations were that the GH pulse signal caused oscillating insulin requirements (Figure 6.6c). Oscillatory messages are common in physiology. Studies have shown well described oscillations in the insulin/glucose homeostatic mechanism with periods ranging from seconds to hours depending in part on their sampling intervals and duration of the study. Insulin release is a complex oscillatory process with rapid pulses superimposed on slower circadian oscillations, but the exact negative feedback loop linking glucose and insulin secretion remains unclear. In the basal state, the plasma concentrations of insulin and C-peptide oscillate about a mean value in monkeys and man with a periodicity of 10-13min (Lang 1979). Oscillations with periods of greater than one hour have been described in the fasting and glucose-stimulated dog (Vanholder 1980). Stimulatory effects of sleep on insulin secretion are achieved by an enhancement of the oscillation amplitude that could be partly mediated

by GH. These different groups of oscillation periods probably reflect separate homeostatic mechanisms with different time courses. Their physiological importance and metabolic consequences are of emerging interest.

To examine if a temporal relationship existed between the GH pulses and the oscillating insulin requirements seen in my overnight studies, cross-correlation analysis was carried out. In my studies maximum insulin requirements lagged behind peak GH levels by 135min (**Figure 6.6d**) but the change was evident by 120min and lasted 240min. Similarly, Moller (1992a) in a study of GH pulse on forearm substrate fluxes in humans showed a change in glucose uptake from 10min which was sustained for 240min and maximal at 120min. Levels of GH achieved ($21\mu\text{g/L}=42\text{mU/L}$) were slightly higher than those in my study (31.5 mU/L). However, this author could not support a primary role for surges of GH (up to $22 \pm 2\mu\text{g/L}$, $44 \pm 4\text{mU/L}$) in the pathogenesis of the dawn phenomenon (Moller 1992b). Their diabetic subjects were studied in the post absorptive state after an iv bolus of GH with isotopically measured glucose turnover and forearm glucose uptake and other factors require consideration.

Fowelin and colleagues (1991, 1995) also showed that whereas lower GH infusion rates provoked a rise in insulin requirements which were maximal between 3 and 4 hours similar to my observations, higher GH infusion rates induced changes which lasted up to 6-7 hours. Maximal effects were seen with plasma levels of 50 mU/L . Levels far in excess of these are often seen in adolescents with Type 1 diabetes. Thus as insulin efficacy is determined by amplitude, GH effects must also result from a modulation of the oscillation amplitude (Song 2000).

Matthews (1983a) showed that pulsatile insulin has greater hypoglycaemic effect in normal humans than continuously administered hormone and suggested that this effect might result from a greater insulin receptor binding. Komjati, 1986 observed a greater efficacy of pulsatile versus continuous insulin delivery in restraining glucose production from the isolated

perifused liver and Bratusch-Marrain (1986a) showed that in insulin-dependent diabetics that pulsatile insulin delivery of a dose less than 40% has an equipotent effect on reducing hepatic glucose production as continuous administration.

In contrast, studies reported by Verdin, 1984, using the euglycaemic-clamp technique combined with 3-[3H]glucose turnover methodology, pulsatile insulin (resulting in peripheral plasma insulin levels oscillating between 10-45 mU/l) and continuous insulin administration (mean plasma insulin levels of 29 mU/l) exerted similar metabolic effects. In vitro studies have shown that after insulin receptor binding and internalisation into cells, insulin and the enzymatic processes involved in its degradation, can also influence the generation of insulin action (Seta 1997).

Matthews, 1990 has used cross correlation to demonstrate that the nadir of blood glucose occurs on average 45 minutes after an increase in insulin rate and that the maximal effect on rate-of-change of glucose occurs after 15 minutes. The 45minute nadir is in accord with the human feed-back loop kinetics of the pancreatic-liver interactions, the 'hepa-beta' loop that oscillates with a period of 40-80 minutes ((Matthews 1983b). The physiology of insulin secretion in normal man, which shows pulsatility with a period of 13 minutes (Lang 1979) and the rapid oscillations of insulin secretion seem to be an inherent feature of the cellular mechanisms of insulin secretion since they persist in the isolated perifused pancreas and in perifused islets. In my studies, the 135min oscillation in insulin requirement induced by GH pulses would be a signal appropriate to the harmonics of both the physiological insulin and glucose oscillations previously seen.

In Type 1 diabetic subjects, as increased GH pulses are prominent both during the day and after the onset of sleep, the endogenous GH pulses would continuously induce a refractory state of its insulin-like effects throughout the day and night. Therefore, studies describing the 'dawn phenomenon' as "diminished insulin sensitivity" (or increased insulin requirements) at specific

time periods (120-240mins) after a GH pulse may be a more appropriate standard convention for subjects in research studies.

Theoretically, the need for insulin could be attributable to an increase in plasma insulin clearance or a decrease in insulin sensitivity or even a combination of both. Some investigators have suggested that an increase in the metabolic clearance of insulin in the early morning, rather than a decrease in insulin sensitivity, is the primary cause of the dawn phenomenon (Arslanian 1992).

Insulin clearance rates in patients with Type 1 diabetes are reported as decreased (Hachiya 1987) or increased (Waldhausl 1985) or unchanged (Navalesi 1978) when compared with healthy control subjects.

When I examined the metabolic clearance rate for insulin in my subjects (Table 6.5a), there was no significant difference between control and continuous GH nights (mean 5.1 and 5.6 mL/kg/min respectively) and only slightly increased during pulse GH at 6.5 mU/kg/min. Thus the changes in insulin requirements that I observed during pulse GH administration must primarily reflect a decrease in insulin sensitivity.

It is interesting to note that the overnight changes in insulin sensitivity seen during my overnight studies are short lived. In the studies of Perriello (1990), the difference in insulin action in the interval of 2400-0200, compared with 0600-0800, tended to disappear during the second hour of the clamp experiments. Under SRIH and euglycaemic clamp conditions in healthy volunteers, the presence of higher plasma insulin levels (80 ± 6 vs. 39 ± 5 mU/L) caused the expected stimulation of both glucose oxidation and non-oxidative glucose uptake (Ferrannini 1989). Perriello (1990) showed that insulin sensitivity decreased at dawn compared to the early night hours attributed to mainly 30% increase in the rate of hepatic glucose production and 25% decrease in rate of glucose utilisation, there being no changes in plasma insulin clearance. In fact, the studies of Widmer, were performed in hyperglycaemic conditions (plasma glucose 7mmol/l) and it is well known that hyperglycaemic conditions

can compensate for impaired insulin action by a mass-action effect (Gottesman 1984). Thus, the increased insulin requirements at dawn must be solely attributable to a decrease in insulin sensitivity at this time of day (Bolli 1988).

More interestingly, a clear temporal relationship between the GH administered at 180-minute periodicity and the change in insulin requirement was noted in my studies. There was no relationship identified between other metabolic parameters and insulin requirements. During my studies in Chapter 6, the pulse GH caused a rise in IGF-1 levels in the 4 subjects analysed, however in these respective subjects, IGF-1 levels did not change during 12 hour continuous GH infusion. Although IGF-I levels were higher on a pulse night, insulin requirements were higher too and ketones and free fatty acids were stimulated. As the time response of IGF-1 required is long, IGF-I could not have contributed directly to the metabolic changes that I observed following GH pulses. To support this further, a study using the hyperinsulinaemic euglycaemic clamp, insulin produced short-term increases in IGF-1 levels in the absence of a GH response (Grant, 1989). Therefore insulin changes purely may have accounted for the rise in IGF-1 levels seen on pulse GH night when insulin infusion rates were higher than on either continuous GH or control nights.

Although in normal subjects, the nocturnal rise of GH did not appear to affect carbohydrate tolerance the following morning (Nielsen 1998), my observations in Type 1 diabetic subjects suggest that a decrease in insulin sensitivity is induced by nocturnal surges of GH secretion. The evidence from my studies suggests that the GH pulse signal is directly responsible for inducing insulin resistance. My studies confirm that GH pulses (achieving peak plasma GH of 26.5-36.9 mU/L) provoke a rise in insulin requirements that are maximal 3 to 4 hours later. Other authors have shown that GH also has an amplitude mediated effect on insulin resistance in subjects with Type 1 diabetes (Fowelin 1995) which induce changes lasting up to 6-7 hours. Furthermore, when examining GH secretion, adolescent subjects with Type 1

diabetes have a shorter GH pulse periodicity than matched controls (Chapter 4) suggesting an increased number of GH pulses.

Given that the number and amplitude of GH pulses overnight vary in many young people with diabetes, I would postulate that a greater pulse frequency would lead to a gradual incremental rise of insulin resistance throughout the night with maximal effects in the early morning. The effect of overnight acquisition of insulin resistance, by a combination of GH pulse amplitude and frequency, is the summation seen in the dawn hours. The differential effects of GH peak and pulsatility would partly explain the variable observation of the dawn phenomenon in Type 1 diabetes.

The Pulse GH Signal (amplitude and frequency) induces an oscillatory rise in insulin requirements causing acquisition of overnight insulin resistance.

(iii) Metabolites

In healthy normal subjects made insulin deficient by somatostatin infusion, when GH was administered blood glucose became grossly elevated along with plasma free fatty acid, ketones and glycerol levels (Metcalf 1981). Similarly, when diabetic subjects, who had euglycaemia maintained with insulin infusion, were changed over to somatostatin and GH infusions, after only six hours, the GH concentrations became elevated and plasma ketone increased impressively while blood glucose remained unchanged (Gerich 1976).

(iv) Ketones

Whereas insulin sensitivity was related solely to pulse GH administration, the effects on β OHB and acetoacetate were evident on *both* GH nights but were more marked on continuous GH nights (section 6.0.5; **Figure 6.6e**, **Table 6.5c**). Two-way Anova on log-transformed data confirmed that ketone levels changed with time between (20.00-08.00h) on all study nights ($F\ 5.95, p < 0.001$) but had

reached plateau levels between 01-08.00h (F 0.7862, p =NS). The mean ketone levels were significantly different between the three study nights (F 83.56, p <0.001) being most pronounced between continuous GH and control (F 111.73, p <0.001). High variation of ketone data was noted between subjects on pulse GH nights.

In previous studies Edge, 1993 noted that the overnight rise in β OHbutyrate in diabetics during an euglycaemic clamp correlated greater with basal rather than with pulse GH. However, Jorgensen, observed no differences between β OHbutyrate after pulsatile or continuous delivery of GH to GH deficient subjects but insulin and glucagon levels were not controlled in their studies (Jorgensen 1990).

Normally, if sufficient insulin is available, much of the ketogenic activity of GH is suppressed (Gerich 1976). However, in my studies despite using a variable-insulin clamp that allowed optimum delivery of insulin to maintain euglycaemia throughout the night, GH signalled a rise in ketone levels, which remained elevated. As would be expected from the sensitivity of lipolysis to insulin, total ketone concentration initially fell rapidly (1900h) and sooner than the glucose concentration. However, following GH administration plasma ketones rose steadily from approximately 21.00h (1 hour after continuous GH was commenced) and around 23.00h (1 hour after first pulse GH given) until peak levels were reached between 01.00-02.00h (3-4 hours after commencing GH). The level of ketones were sustained at this high level until 08.00h (**Figure 6.6c**) and the mean fasting (08.00h) total ketone levels were elevated (Control 209.9; Continuous GH 517.6; Pulse GH 380.8 μ mol/L). The fasting ketones were particularly marked during continuous GH administration and of similar magnitude to that reported in other studies (500 μ mol/l) in diabetic subjects.

However, the overnight pattern reported in previous studies is a rise of ketones around midnight followed by a fall around 04.30h (Wildenhoff 1972, Edge 1993). I noted no reversal even on pulse nights, although ketone levels

were measured hourly. Furthermore, during studies in forearm substrate when a GH bolus is administered, an increase in β OHB occurs maximal at 2h and reversed by 4h (Moller 1990). In addition, a 70% increase in serum NEFA is seen after 180min and a 400% increase in 3-OH butyrate after 240mins (Moller 1992a). The pattern I observed was a consistent rise of ketones following GH administration and persistence of elevated levels after 01.00h to the high fasting levels seen at 08.00h. This appears to be directly triggered by GH alone in contrast to control study night.

Part of the explanation for the higher ketone levels noted on continuous GH nights may simply be due to the comparatively higher insulin levels measured on pulse GH nights. Sherwin (1976b) identified a 42% reduction of metabolic clearance rate of ketone bodies in diabetic subjects compared to control that may also contribute to hyperketonaemia. This reduced clearance reflects the progressive saturation of muscular uptake that occurs with increasing ketonemia. The hormonal and metabolic environment appears to play only a minor role in this process.

During progressive fasting in normal subjects, the production rate and concentrations of ketones rise during the early phase of fasting and reach a plateau after 5 days (when GH is maximal). The mechanism appears to be that as extraction of ketone bodies by muscle becomes limited during ongoing fasting, ketone are preferentially taken up by the brain to serve as a substrate replacing glucose. A negative feedback mechanism exists whereby ketone bodies restrain their own production rate through their antilipolytic and insulinotropic effects (Moller 1990). Thus, ketones are homeostatically maintained only slightly above the maximal metabolic disposal rate, and the difference corresponds to the urinary excretion (Balasse 1989).

When Type 1 diabetic subjects are compared with fasting control subjects, some authors report that the ketone body kinetics are comparable and the maximal removal capacity is identical in the two situations (Fery 1985, Balasse 1989). However, when evaluating ketone body utilization in the muscle of fasted and

streptozotocin diabetic rats, there was a markedly lower muscle enzyme activity of 3-oxoacid CoA transferase which prevented ketone body uptake in diabetic rats (Okuda 1991) compared to fasted rats. The presence of raised ketone levels during either situation inhibited both glucose utilization and decreased the phosphofructokinase activity in muscles (Okuda 1991) encouraging oxidation of ketones as an energy source rather than glucose. This provides a mechanism for ketoacidosis. Hyperglycaemia, through substrate competition at the acetyl-coenzyme A level, could also theoretically decrease the disposal rate for ketone bodies. However, even during hyperglycaemic clamp conditions in subjects with Type 1 diabetes, no inhibitory role for hyperglycaemia in the disposal of ketones was identified (Moller 1990). These data therefore suggest that an *excessive production* of ketone bodies is the cause of persisting uncontrolled hyperketonaemia. A production even slightly above that in prolonged fasting is sufficient to cause a progressive build-up in concentration because of reduced ketone body uptake by muscle (reduced 3-oxoCoAT and saturation-limited). The oxidation of ketone bodies inhibits phosphofructokinase activity and glucose utilization in muscle. The mechanism of increased ketones seen in my studies therefore appears to be that GH is directly responsible for the initial excessive production of ketones. When clearance of elevated ketones is limited by saturated muscle extraction, this leads to a progressive build-up manifesting in sustained and elevated levels throughout the night.

Recent concern is the role that ketones may have on the brain. When measuring brain β OHB and lactate in fasted adults, plasma and brain β OHB correlated well ($r=0.86$) (Pan 2000). This implies that the influx of ketone bodies into the brain is largely determined by the amount of ketones present in the blood (Hasselbalch 1995) which is of great concern when ketones rise above normal in Type 1 diabetes. Furthermore, recent work in mouse brain suggests that ketones may directly affect the blood-brain barrier impermeability as they have shown that β OHB can increase vascular permeability factor in endothelial cells and acetoacetate increases the production of the potent vasoconstrictor,

endothelial-1 (Isales 1999). These effects have been noted clinically in paired control experiments in normal volunteers where infusions of β OHB and lactate increased the threshold for initiating hypoglycaemia and reduced the magnitude of autonomic and neuroglycopenic symptoms, poor counter-regulatory hormone responses and cognitive dysfunction (Veneman 1994). Elevated ketones may be responsible for the defective counter-regulation causing prolonged nocturnal hypoglycaemic episodes reported in children with diabetes (Matyka 1999).

(v) Lactate

Lactate is an indicator of cellular energy metabolism. Normally an increase in lactate results from ketones displacing lactate oxidation without altering glucose phosphorylation and glycolysis, but I observed a fall of lactate: Concentrations fell during the night on all three occasions but were slightly higher on pulse GH night. During normal activity in Type 1 diabetes blood lactate levels are usually below 2mmol/l (Alberti 1975).

Oscillatory organisation between insulin, insulin sensitivity, glucose and lactate were examined in 24-hour profile from healthy volunteers (Feneberg 1999). Lactate fluctuations exhibited pulsatile fluctuations at an average of 73-95minutes during the day and night, and showed temporal and pattern synchrony with insulin pulses (periodicity of 75-97minutes) and this was inversely correlated to insulin sensitivity. I could not detect any oscillation with lactate levels, apart from a mild elevation towards the early morning on a pulse GH night when there was changing insulin sensitivity.

Blood lactate may rise in response to decreased hepatic uptake for gluconeogenesis or increased production. Following insulin, hepatic uptake does not change but gluconeogenic precursors are diverted into glycogen. As optimal insulin replacement was provided and euglycaemia maintained, this might account for the lower levels of lactate measured.

(vi) NEFA

Lipolysis is very sensitive to low concentrations of insulin and it was surprising to record during my overnight euglycaemic clamp studies, when optimum insulin was supplied, that both ketones and NEFA rose in response to GH (Figure 6.6e).

A recent study by Avogaro 1992 looking at the effects of low (20 μ U/ml and 35 μ U/ml) and high (70 μ U/ml) plasma insulin concentrations on ketone and lipolytic responses when stimulated by adrenaline in Type I diabetic patients, showed increased ketogenic response and normal NEFA response at low insulin levels but high insulin levels were able to suppress ketogenic responses. My overnight studies showed moderate levels of plasma insulin levels during euglycaemic clamp (pulse GH night: 26.53 ± 3.01 mU/L vs. control 17.16 ± 0.42 ($p=0.01$) and continuous GH 17.88 ± 1.55 mU/L ($p=0.01$) yet a rise in free fatty acids were seen on both GH nights.

During my studies, mean (\pm SEM) plasma free fatty acids were increased primarily during continuous GH (1.09 ± 0.07 mmol/L) compared to both control (0.61 ± 0.05 , $p=0.001$) and pulse GH (0.94 ± 0.04 , $p=0.003$) suggesting that a raised continuous level of GH is the primary message that stimulates lipolysis.

Previously, authors have considered that a direct lipolytic effect of GH is not seen in human fat cells in-vitro (Goodman 1970). In contrast, other workers have shown that a GH pulse of 200microgram (=400mU) can induce peak interstitial glycerol levels locally in adipose tissue after 135min (femoral adipose) and 165min (abdominal adipose) which is not reflected in plasma glycerol (or NEFA) level changes (Gravholt 1999). This also confirms the direct action of GH pulse on lipolysis.

Along with these observations, in vitro studies of the actions of GH in adipose cells initially produced conflicting results of a lipolytic effect (Richelsen 1997, Carrel 2000). Further studies in cultured 3T3-adipocyte cell line, which provides an appropriate model for the study of short and long-term effects of

GH, indicate that GH has a dual effect on adipocytes depending on the duration of exposure. Acute exposure increased glucose uptake and utilisation by 3T3 adipocytes, whereas an extended incubation of 24-48 hours induced a suppression of glucose metabolism (Schwartz 1985). Other studies indicate that GH modulates the lipolytic actions of other stimuli and has a modest direct lipolytic activity (Berneis 1996, Goodman 1991). In contrast in one study, a short incubation with GH produced a transient antilipolytic effect (Dietz 1991). The mechanisms underlying the dual effect of GH are presently unknown.

Experiments in rats suggest that NEFAs reduce hepatic insulin uptake and insulin degradation (Svedberg 1990). In humans NEFAs have been shown to alter hepatic insulin clearance and have been implicated in the reduced insulin sensitivity associated with Type 2 diabetes (Boden 1997). More recently, NEFAs also have been thought to regulate hepatic glucose metabolism and mediating the actions of insulin on the liver (Cherrington 1998, Piatti 1999).

Lipid-induced insulin resistance has been investigated in normal subjects using euglycaemic and hyperinsulinaemic clamp, with or without heparin-induced lipolysis after infusing triglyceride emulsion compared to control. Peripheral insulin resistance was observed in the presence of a combined increase in total lipid and NEFA oxidation, but not during an isolated increase in NEFA oxidation whereas hepatic insulin resistance could be induced even by a moderate increase in NEFA availability (Laville 1995).

Studies on forearm glucose uptake shows that the activity of the glucose-fatty acid cycle is stimulated at physiological plasma NEFA levels at both hyperglycaemic and euglycaemic blood glucose concentrations but NEFA activity causes a decrease in peripheral insulin sensitivity only during euglycaemia (Walker 1991). Although NEFA levels were higher than control on both pulse and continuous GH study nights, insulin requirements only changed on pulse GH night.

During my studies glucagon was infused on both study nights. Chambrier, (1990) in their study of the interactions of glucagon and free fatty

acids with insulin on glucose production and utilisation in normal subjects infused with somatostatin, showed that the maintenance of glucagon level had only small and inconsistent effects on glucose utilisation but induced a shift to the right of the dose response curve to insulin of endogenous glucose production (ED50: SRIH+insulin 10.9 mU/l; SRIH+insulin+glucagon 15.2 mU/l). Addition of intralipid infusion resulted in almost total suppression of the insulin-induced increase of glucose utilisation whether glucagon was substituted or not. In the absence of glucagon, intralipid infusion antagonised the action of insulin on endogenous glucose production, however this effect was no longer apparent when glucagon was replaced. This may explain my studies as I did not observe an association between NEFA levels and insulin requirements on either GH nights.

Irrespective of mechanisms, the elevated NEFA levels may lead to a decreased glucose utilisation during moderate hyperinsulinaemia and thought to mediate the insulin resistance in Type 2 diabetes (Bergman 2000a).

In 1965 Randle demonstrated that fatty acids compete with glucose for substrate oxidation in isolated rat heart muscle and rat diaphragm muscle. This model predicted an increase in muscle glucose-6-phosphate and mechanisms that suggested a limitation of glycolysis (Randle 1998). More recent studies using euglycaemic, hyperinsulinaemic conditions with either high or low levels of plasma fatty acids, showed that by increasing plasma fatty acid concentration for 5 hours, this caused a reduction of approximately 50% in insulin-stimulated rates of muscle glycogen synthesis and whole body glucose oxidation compared to normal control subjects and found a fall in intramuscular glucose-6-phosphate (Roden 1996)

This suggests that increases in plasma fatty acid concentrations initially induce insulin resistance by inhibiting glucose transport or phosphorylation activity and that reduction in muscle glycogen synthesis and glucose oxidation follows (Dresner 1999).

The complex GH rhythms of diabetes mimic those of fasting where a sustained rise in free fatty acids is also seen with the change in GH pattern (Ho 1988). Some recent work in animals show that the influence of dietary intake level alters protein (leucine) and palmitate/NEFA flux rates to a GH bolus. Animals fed on the lowest intake (0.8 of maintenance energy requirement given hourly) showed an immediate response to GH (within 3 hours of administration) with increased plasma NEFA. However, appropriately fed (1.2-2.65 x maintenance energy requirement) showed a markedly reduced response that was not apparent for 22-24 hours (Dawson 1998).

As lipid metabolism is differentially responsive to GH and nutritional status, the endocrine adaptation in diabetes parallels the physiological state of "starvation" inducing exaggerated NEFA responses to GH pulses.

Overnight GH either administered as a continuously elevated GH (8.6-10.8mU/L) or discrete 60minute pulses of GH (26.5-36.9 mU/L) will initiate ketogenesis and lipolysis that contributes to the metabolic deterioration in subjects with Type 1 diabetes. Pulses of GH independent of other mediators induce peripheral insulin resistance.

CHAPTER 7

INTEGRAL MESSAGES: FINAL CONCLUSIONS

INTEGRAL MESSAGES: FINAL CONCLUSIONS

Previous theories related metabolic problems in adolescents with Type 1 diabetes simply to hyperglycaemia. A number of altered metabolic states such as insulin resistance, increased ketones and fatty acids and elevated GH are now implicated.

During puberty GH pulses are often exaggerated (peak amplitude, mean and trough) in Type 1 diabetes compared to control subjects. I have shown that a combination of decreased GH clearance and increased GH secretion exists in adolescents with Type 1 diabetes. Part of the neurosecretory alteration causing increased GH secretion appears to be that an increased cholinergic tone is present causing increased peak GH responses to GHRH stimulation. Of greater interest is the cause of the raised trough GH level, which is determined by alterations in SRIH tone. I have proposed the hypothesis that hyperinsulinaemia may antagonise somatostatin suppression of GH, contributing to the higher mean and trough GH levels seen.

Disorganised or elevated serum GH concentration profiles with alterations in trough concentrations are characteristic of the physiological adaptation in fasting or starvation (Ho 1992, Hartman 1996). Elevated GH levels are seen in other pathological models of nutritional deficiency, such as anorexia (Laughlin 1996, Stoving 1999). As oscillatory organisation is an universal mode of signal transduction representing major functional process in biological rhythms, there should be a rational basis for this type of GH secretory profile. Therefore when searching for the reason and cause of the GH pattern alteration in Type 1 diabetic subjects, the evidence may be found within the model of fasting.

Starvation-induced enhancement of GH production rate in normal healthy adults is seen after two days of fasting and is related to an increased GH

burst frequency, increased GH amplitude and shorter periodicity (fasted subjects 88 ± 4.2 versus fed subjects 143 ± 14 minutes) (Hartman 1992, Bergendahl 1999). I noted a similar alteration in GH periodicity when examining spontaneous GH secretion in adolescent subjects with Type 1 diabetes compared with controls (normal puberty-matched).

Exploration of the signals for cell metabolism suggests the pattern of pulsatile release of a hormone may code important signals for their target organs (Pau 1997). Pulses of insulin are certainly more efficacious in altering glucose flux. I have shown that the GH pulse signal is solely responsible for the recruitment of insulin resistance overnight and postulated that overnight insulin would rise incrementally either with a changing GH pulse amplitude or altered GH pulse frequency. The 90-minute GH periodicity of secretion found in my diabetic subjects in Chapter 4 and seen during starvation would be an appropriate harmonic for the oscillatory release of insulin from intact beta cells of the islets, when these are present and responsive in the functioning pancreas.

My studies from Chapter 6 confirm that even during euglycaemia and adequate insulin supply (during clamp conditions), the decreased insulin sensitivity seen in adolescents with diabetes are caused directly by the pulses of GH. From my studies the GH pulses given overnight at 180-minute cycles, induced a pulsatility of insulin resistance that occurred with a lag phase of 135minutes. As insulin requirements oscillated during these *glucose-clamp* studies, were insulin requirements to remain stable, a rise and fall of blood glucose in an oscillatory fashion would be observed.

In-vitro studies suggest that spontaneous fluctuations of intracellular energy metabolism also occur with oscillatory changes of glucose. Recent work has shown that in vivo oscillations in glucose at significantly high doses of 6 mg/kg/min for 10 minutes caused synchronised pulsatile insulin release in normal subjects. Glucose flux may be an important regulator of pulsatile insulin secretion in normal subjects. Thus, intermittent GH pulses, evokes oscillations in glucose (or insulin requirement) of 135minutes, which would

stimulate recurrent insulin secretory signals at the phase-harmonic of 45mins, as that reported for insulin action. It appears that during starvation pulses of GH at 90minute periodicity occur to induce alterations in glucose and insulin flux for energy utilisation. In Type 1 diabetes this manifests as insulin resistance and rising glucose levels in the face of depleted or inappropriate timing of insulin replacement.

The optimum duration of the pulsatile signal and the optimal interval between successive pulses vary as a function of the rates of receptor desensitisation or resensitisation and of the maximal ligand level during stimulation (Goldbeter 2000). GH has a changing half-life critically dependent on duration of exposure following an asymptotic relationship. I have shown that in adolescents with Type 1 diabetes this half-life is prolonged compared to controls at all exposures, and reaches a maximum half-life of 28.5minutes above 60minutes GH exposure. Neither short-term hyperglycaemia, normoglycaemia nor hyperinsulinaemia affect these GH decay characteristics.

Studies in the rat have established that the pattern of GH administration is a significant determinant of GH receptor status. Maiter (1988) reported that hepatic receptors of hypophysectomised rats were upregulated when the hormone was administered as a *continuous infusion* but not as multiple subcutaneous injections. Furthermore, these investigators have reported similar changes in serum GHBP activity in rats from the same study, that is an increase in GHBP activity occurred only with continuous administration (Maiter 1992). These animal data raise the question as to whether GHBP levels in man might also be modulated by the pattern of delivery.

There is a major difference regarding the origin of GHBP in rat and man. In the rodent, GHBP and the GH receptor have been shown to be separate products of two distinct messenger RNA's derived from a single gene encoding the GH receptor (Baumbach 1989, Smith 1989b). However GHBP mRNA but not GH receptor mRNA was increased by continuous GH infusion suggesting that the effects of continuously elevated GH on GH

receptor and GHBP gene expression have differential effects at transcriptional and translational level (Maiter 1992). In fact the rat's insulin is directly involved in the GH receptor expression (Chen 1997). A separate transcript for GHBP has not been reported in humans and therefore this protein is likely to be derived from proteolytic cleavage of the GH receptor (Baumann 1988). Consequently GHBP levels in man reflects a parallel change in GH receptor status. More recent work has allowed the determination of GHBP in plasma, which is reported with a half-life of 30mins and suggests that the GHBP is the critical limiting ligand for GH clearance. This is consistent with the maximum half-life of GH measured in my diabetic subjects in Chapter 3. Thus, the GH clearance mechanism is fully saturated and maximised in diabetic subjects.

Although muscle could be a potential tissue for GH clearance as the presence of GH receptor mRNA in skeletal muscle is established, most investigators have been unsuccessful in demonstrating any specific binding of GH to skeletal muscle or to myoblasts in culture. It has been equally difficult to show direct actions of GH on cultured muscle cells; GH stimulates expression of IGF-I genes in skeletal muscle, although there are a number of cases in which skeletal muscle IGF-I expression is elevated in the absence of GH. The actions of GH on muscle may thus be predominantly IGF-1 mediated. The only positive report concludes that the early insulin-like effects of GH can result from direct interactions between GH and isolated muscle cells (Florini 1996).

During starvation, a possible mechanism might be that the growth-promoting actions of GH are reduced as fuel must be diverted towards nutritional energy need. The *continuously raised GH level* may upregulate GHBP (hence desensitises GH receptors) resulting in a relative GH resistance. The mechanism in Type 1 diabetes could parallel starvation and as such, this GH receptor resistance, delays clearance compounding the high GH levels already present. The elevated GH in diabetic subjects may be due to hepatic resistance to GH induced by the presence of continuous GH and saturated

GHBP. Reduced IGF-1 generation together with increased hepatic production of IGF-1-binding protein-1 (IGFBP-1) leads to reduced levels of circulating IGF-1 and further stimulation of GH production. Increases in GH secretion and serum IGFBP-1 may compound the problem of imperfect insulin replacement both by inhibiting insulin action and by reducing available insulin-like activity. Thus, the signal of a continuously raised baseline GH downregulates its own receptors causing GH resistance, further stimulating GH release and worsening insulin resistance.

The continuous signal diverts GH action towards 'nutrition conservation' as during starvation. My observations suggest that a raised GH of 8.6-10.8 mU/L provides a continuous GH signal to enhance ketones, both from hepatic induction and stimulation of lipolysis. The picture of insulin resistance is made more complex by these metabolites. Patti et al (1999) have shown that nutrients (fatty acids or amino acids) inhibit early post-receptor steps in insulin action both in vitro and in several in vivo models. These data suggest that nutrients directly modulate insulin signalling, perhaps via common pathways with GH, and contribute to cellular insulin resistance experienced by diabetic subjects.

Marked hyperketonaemia was seen on both GH nights compared to control study night. Although in healthy subjects hyperketonaemia has been shown to have no effect on basal glucose production or the suppression of hepatic glucose production following hyperinsulinaemic euglycaemic clamp (Bratusch-Marrain 1986b), experiments in the rat show that after prolonged ketone exposure there is a depression of insulin-stimulated glucose oxidation which lasts for 4 days (Skutches 1990). This implies that the prolonged insulin resistance seen in fasting and Type 1 diabetes could also be attributed to the metabolic influence of ketones. In a further study, elevation in ketone levels in dogs was shown to directly affect glucose and free fatty acid metabolism independent of changes in insulin and glucagon levels or when sympathetic activity was blocked by α and β blockade (Shaw 1984). However, work in the

rat pancreas has shown that perfusion with only *supraphysiological* concentrations of β OHB and acetoacetate (10 mmol/L) produced increases of insulin release of any significance during normoglycaemia, but lower physiological levels of ketones (0.1-1mmol/L) did not stimulate insulin release. This suggests that in diabetic subjects that the ketones levels observed in my studies would not alter insulin requirement. Furthermore, as ketones at both levels do have a direct inhibitory effect on glucagon secretion from perfused rat pancreas (Ikeda 1987), the role of ketones is mainly through their action on depressing glucagon. I thus conclude that in my subjects with Type 1 diabetes, raised ketones, although are important in contributing to impaired response of glucagon to hypoglycaemia, is unlikely to influence insulin-resistance seen in my studies.

Non-esterified fatty acids (NEFA) act as an important signal as well as a metabolic substrate. Numerous data now suggest that non-esterified fatty acids (NEFA) can act without any metabolic modification as second messengers or modulators of the complex signalling network, and include amplification, inhibition or signal redirection affecting molecular, tissue and organ situations (Nunez 1997). For example, it has been considered that the primary route by which insulin maintains control over glucose production is indirect and is mediated by the regulation of non-esterified fatty acid release from the adipocyte (Bergman 2000b). The glucose-stimulated insulin secretion is ablated in fasted rats when an antilipolytic agent, nicotinic acid, is administered concurrently but unaffected in fed rats suggesting a powerful interaction between glucose and fatty acids (Stein 1996).

My observations confirm that the insulin requirements changed *only* on pulse GH study night but an increase in NEFA was seen on both GH nights, with a higher measured NEFA level on continuous GH night. My conclusion is that insulin resistance seen overnight was therefore unlikely to be related to changes in NEFA. I propose this was elicited directly by GH. Furthermore,

the phase-lag of insulin resistance oscillated with the GH pulse while the pattern of NEFA changes was unrelated to this insulin resistance.

In support of my findings, a recent report suggests that free fatty acids also oscillate frequently 6-8.5mins but this was *independent* of any insulin oscillations and more likely to be central nervous system controlled (Getty 2000). In contrast to my findings, studies in healthy adults during euglycaemic hyperinsulinaemic clamp show that GH mediates the reduction of insulin effect on the liver, but GH action on glucose and lipid oxidation is *indirectly* mediated by its lipolytic activity (Piatti 1999).

NEFA can regulate glucose utilisation in muscle and are important signals to the liver and intact beta cells. To evaluate whether stimulation of lipolysis affects the induction of insulin resistance by GH, studies using Acipimox, a nicotinic acid analog that inhibits lipolysis, show that when basal rates of lipid oxidation were suppressed, basal glucose oxidation rates were lower with GH alone compared with GH+acipimox. This suggests that the insulin antagonistic actions of GH are causally linked to the activation of lipolysis (Nielsen 2001). However, Nielsen (2001) also noted that GH may induce some residual insulin-resistance through a non-NEFA-dependent mechanism.

Other studies in the rat have shown that GH infusion did not alter free fatty acids or insulin levels but in contrast, decreased insulin-stimulated glucose uptake by 32%, glycolysis by 27% and glycogen synthesis by 40% (Kim 1999). The suppression of glycogen synthesis implies that metabolic impairment precedes and causes development of peripheral insulin resistance, independent of either free fatty acids or insulin. These findings and my observations suggest that GH has a direct cellular and molecular mechanism eliciting the insulin-antagonistic effects of GH which are *independent* of both plasma free fatty acids and insulin, but the role of fatty acids takes effect in certain metabolic circumstances.

In vivo administration of GH induces lipid oxidation and enhances lipolysis but it is unclear whether GH directly promotes this or increased NEFA levels drive it. Interesting work by Leung (1997) using an in-vitro bioassay for assessing beta-oxidation of fatty acids in mitochondria, found that GH directly stimulated fatty acid oxidation, whereas large doses of IGF-I (up to 250 nmol/l) had no effect. Further evidence is emerging about the direct lipolytic effect. The 20K hGH might show higher lipolytic activity than 22K hGH in adipose tissue, particularly as 22K hGH produces a large amount of GHBP (Asada 2000). Analysis on inhibition and phosphorylation of signalling molecules suggested that GH-induced lipolysis stimulation is dependent on gene expression and mediated through a pathway which, when activated, continues for 48 hours (Asada 2000). These data suggest that GH activates lipolysis and NEFA release, but continuously raised NEFA levels may be promoted by a different mechanism.

The effects of insulin on serum lipids as a substrate are complex. Insulin normally inhibits adipose tissue lipolysis and decreases free fatty acid into the plasma. Schade and Eaton (1977) showed that glucose production and lipolysis were suppressed at a dose of insulin of only one-tenth that needed to stimulate glucose uptake. There is however, stimulatory effects of insulin on hepatic lipogenesis and esterification of free fatty acids to form triglycerides. The metabolic processes are thus intricately influenced by different concentrations of insulin. Independently GH triggers both lipolysis, releasing free fatty acids, and encourages insulin resistance. Conversely, free fatty acids tend to exert their effects by modulating hormones, particularly insulin, and also ketones, but by a different mechanism (Fery 1996).

The neuroregulation of GH secretion and the state of the adipose tissue reserves are also closely related. The mediators of GH regulation exerted by the adipose tissue are not clearly understood, but both free fatty acids and adipocyte-produced hormone leptin have been identified (Pombo 1999, Astorga 1999). Experiments conducted to determine the effect of free fatty acids and

glucose treatment on GH and LH secretion in the pig suggested that the number of GH pulses were only increased by free fatty acids infusion. This indicates that free fatty acids is likely to be a more effective modulator of GH secretion than acute hyperglycaemia and may account for the change in GH pulsatility during nutritional conservation.

A rise in free fatty acids normally blocks GH secretion. This action is rapid, dose-related and exerted at the pituitary level with no evident hypothalamic participation. The adipocyte produced hormone leptin has no direct action on the pituitary and its action at the hypothalamus is mediated by neuropeptide Y which is the final step in reducing somatostatin tone, which in turn would cause elevated trough levels of GH. Thus free fatty acids normally exert an inhibitory feedback action on GH secretion in physiological conditions.

However, evaluation of the starvation model suggests that even in the presence of enhanced lipolysis, high plasma GH levels still exist with enhanced pulsatility. The high GH levels trigger lipolysis and further NEFA release. The mechanism of GH control therefore does not appear to be influenced by an inhibitory feedback from NEFA levels during starvation or in diabetes. This suggests that either that the pituitary is unresponsive to increases in NEFA or pituitary antagonism by another factor exists or that the set point for response is altered.

When trying to resolve this matter, we can look to other studies to provide various pieces of evidence. In subjects with anorexia nervosa, authors have shown that the somatotroph sensitivity to the inhibitory feedback action of NEFA is still preserved (Gianotti 2000). I conclude therefore that despite the markedly raised NEFA levels seen in Type 1 diabetic subjects, NEFA levels are not able to influence GH regulation, with neither a role in GH stimulation or in promoting GH suppression. An alternative factor has a superior influence on GH causing direct pituitary antagonism (pituitary resistance to SRIH) or preventing SRIH action on GH suppression.

Discussions in chapters 4 and 5 arising from my observations suggest that in diabetic subjects a reduced somatostatin tone is present because of cholinergic overactivity. This may persistently exist due to alterations in the expression of SRIH or GHRH mRNA or the pituitary GH-receptor in specific areas in the anterior pituitary as demonstrated in both poorly-controlled or well-controlled diabetic rats (Busiguina 2000).

In contrast with humans, metabolic perturbations such as food deprivation and diabetes mellitus normally lead to GH suppression in the rat, partly due to increased somatostatin tone. A significant reduction (50-80%) in SRIH receptor concentrations occurs in the hypothalamus and anterior pituitary gland within 5-7 days of induction of diabetes in the rat (Pesce 1994, Berelowitz 1995). Hypothalamic and pituitary SRIH receptor levels remain lowered in these diabetic rats despite chronic insulin replacement that restored plasma GH levels (Pesce 1994). Altered pituitary somatostatin receptor expression in food deprivation and diabetes could develop as a result of chronic exposure to increased plasma somatostatin causing desensitization of SRIH receptor binding (Berelowitz 1995, Bruno 1994). These animal observations indicate that pituitary sensitivity to somatostatin may be altered in Type 1 diabetic patients.

The treatment of diabetes with inappropriately high insulin doses may also affect somatostatin sensitivity. Hyperinsulinaemia may arise inadvertently when trying to overcome insulin resistance in the diabetic patient. I have shown that hyperinsulinaemia is correlated with decreased GH suppression in the presence of a constant somatostatin infusion. Insulin is known to be stimulatory to GH production either directly or indirectly. A mechanism can be postulated that links long term changes in SRIH receptors with chronically raised insulin levels. Additive to this is the absence of normal GH negative feedback. GH is known to regulate its own synthesis and release by altering expression of key hypothalamic neuropeptides and by modulating the sensitivity of the pituitary to hypothalamic input by regulating pituitary receptor synthesis. In animal models (the GH receptor/GHBP gene-disrupted

mouse) when IGF-1 levels are low and the circulating GH levels are high because of lack of negative feedback, both hypothalamic and pituitary expression is altered to favour stimulation of GH synthesis and release (Peng 2001). In particular, both hypothalamic and pituitary GHRH was elevated whereas SRIF levels were reduced.

Thus, a complex combination of anatomical and physiological alterations within the pituitary, hypothalamus and receptors take effect early in diabetes, and left uncorrected, become irreversible and hence unresponsive to normal metabolic influences, leading to hypersecretory GH state. Normalisation of glucose has no influence on improving this GH hypersecretion. Raised NEFA does not suppress GH release. Replacement of insulin in the face of persistently raised GH levels leads to the additive role of hyperinsulinaemia. Insulin antagonises SRIF action and may be stimulatory to GH with further exacerbation of plasma GH levels and a deteriorating metabolic state.

The combination of raised GH levels, insulin resistance, hyperinsulinaemia and metabolic alterations with elevation of ketones and NEFA may all influence longer term problems in diabetic subjects. Raised GH levels may be involved in the development of proliferative retinopathy either directly (through its effects on arterial medial cell growth) or indirectly by its production of IGF-1 which has potent mitogenic effect (Pfeiffer 1995). The significance of the GH/IGF system in the development of diabetic kidney disease is also emerging (Flyvbjerg 1997, Baud 1999) requiring to identify new targets for a therapeutic approach in glomerular disease. Acute ketosis is also associated with a disturbance of endothelial function that is independent of hyperglycaemia (Avogaro 1999). Recent studies have suggested that ketones may increase lipid peroxidation (Jain 1999) and this elevated cellular lipid peroxidation may play a role in the development of cellular dysfunction and other complications of diabetes. Furthermore, insulin resistance and poor metabolic regulation consequent to GH hypersecretion are believed to also accelerate the development of diabetic angiopathy (Orskov 1996).

Although GH has long been known as a regulator of body growth and metabolism, its mechanism of action at the cellular level has been elusive. Various signalling molecules, proteins and hormones work together to elicit its diverse effects (Carter-Su 1996, Thomas 1998). This Thesis confirms that the qualitative alterations in the GH pulse signal and the subsequent metabolic alterations seen in the diabetic state are parallel to the alterations seen during human starvation. Metabolic decompensation is thought to contribute to ongoing radical and oxidative stress with the increased risk of complications (West 2000). These combined effects may be deleterious to the insulin-dependent diabetic patient, in whom increased GH may precipitate and maintain acute metabolic derangement and play a role in long term consequences.

Observations in this Thesis postulate the mechanism for the metabolic deterioration seen in adolescents with Type 1 diabetes. Raised GH pulse amplitude and increased GH pulse frequency encourages insulin resistance. As a consequence hyperglycaemia, ketosis and lipolysis ensue, each independently fuelling metabolic alteration. Hyperglycaemia and insulin resistance also encourages the use of large doses of exogenous insulin in an attempt to overcome these problems, leading to a hyperinsulinaemic state. Consequent to this, hyperinsulinaemia antagonises somatostatin suppression of GH, directly or indirectly and this combined with the alterations in somatostatin receptors and cholinergic imbalance, leads to chronically reduced SRIH tone in diabetes. This interaction provides a vicious cycle of insulin-induced insulin resistance, via the promotion of persistently elevated levels of GH.

Unravelling the complex interactions of hormones and metabolites will allow the clarification of the spectrum of distinct metabolic sequelae. The partnership of investigative and therapeutic strategies aims to diminish metabolic complications until a cure is found.

APPENDIX

APPENDIX 1.0

METHODS OF ESTIMATING ENDOGENOUS GH SECRETION

APPENDIX 2.0

PROCEDURES 1: METHODS AND EXCLUSIONS

APPENDIX 3.0

PROCEDURES 2: INFUSIONS AND ASSAYS

APPENDIX 4.0

STATISTICS AND ANALYSES

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INSULIN-VARYING CLAMP

APPENDIX 6.0

RAW DATA

APPENDIX 1.0

METHODS OF ESTIMATING ENDOGENOUS GROWTH HORMONE SECRETION

The pattern of pulsatile release of the hormones may be important as a signal for their target organ. The formulation of algebraically explicit biophysical models of GH secretion and clearance has made possible a complete quantitative description of GH secretory and clearance dynamics. Such analytical tools allow investigators to enumerate with statistically bounded confidence limits the number, amplitude, duration, and temporal locations of all significant underlying secretory bursts and simultaneously calculate the half-life of endogenous GH disappearance from all GH concentrations and their variances considered together. In conjunction with contemporary refinements in GH assay techniques, such novel approaches to dissecting the temporal structure of GH secretion and clearance *in vivo* should result in significantly enhanced understanding of GH dynamics in health and disease (Veldhuis 1988a).

Deconvolution Analysis

I provide the principles of these methods in this appendix should you wish to compare other methods to the model I have chosen to use in my studies in Chapter 3.

A1.1 Method of Thompson (THOM)

This method estimates the total amount of secretion over a period of time without considering the secretory pattern. The theoretical background has been described by Tait since 1963 (Tait 1998) and assumes that metabolic clearance is constant and independent of plasma hormone concentration. For GH, these criteria are assumed to be met (MacGillivray 1970, Taylor 1969, Jorgensen 1989).

The equation is as follows:

$$\text{Secretion (mU)} = \text{MCR (l/min)} \cdot \text{time (min)} \cdot \text{ICGH (mU/l)}$$

The term [ICGH.time] is identical to the area under the plasma concentration curve (AUC), which can be calculated with the trapezoidal rule. As GH clearance has been shown to correlate well with body surface area (MacGillivray 1970) MCR can be expressed in litres/day.m². An MCR of GH of 179 litres/day.m² has been chosen for some studies based on a weighted mean of 5 studies (Taylor 1969, MacGillivray 1970, Thompson 1979, Baumann 1979). Most of these studies were performed in adults, but in children similar values have been found (MacGillivray 1970). BSA can be calculated according to Dubois & Dubois although more recent estimates to improve accuracy have been suggested (Jones 1985).

A1.2 Method of Hellman (HEL) using the Pulsar algorithm

Pulsar - The Pulsar program developed by Merriam & Wachter (1982) was used to detect serum GH concentration peaks. The program identifies peaks by combining criteria of height and duration after subtracting a calculated trend. To account for the assay SD, which is known to be concentration dependent, the individual SD for each sample is calculated using the equation $SD = ax^2 + bx + c$ in which a, b and c are the constants in the regression equation of the assay control series. After subtracting the trend from the data, the residuals are divided by their SD leading to a series of signal-to-noise ratios. These ratios are tested using different cut-off criteria for peaks of different duration. Threshold values (G values) which keep the false-positive error rate on signal-free noise below 5% (Merriam 1982) are chosen. A smoothing time is set, the splitting cut-off parameters and weight assigned to peaks. The number of peaks estimated by PULSAR compared to those found by visual inspection shows an excellent correlation for higher peaks, but small peaks detected visually do not meet criteria of PULSAR (Kuilboer 1992).

HEL

With this method, the amount of secretion necessary to establish a peak concentration is calculated per peak, taking into account the ongoing elimination during the secretory episode. Significant peaks and baseline are estimated using the Pulsar program.

Clearance is assumed to be dependent on body weight and defined as

$$\text{MCR} = (\ln 2 / t_{1/2}) \cdot V$$

in which V is the distribution volume. The distribution volume is calculated as 7% of the body weight (Finkelstein 1972, Refetoff 1970). $T_{1/2}$ of 23.5 min is the mean reported from 3 studies (Refetoff 1970).

The secretion per peak is represented by equation

$$S = \frac{V \cdot [C_1 - (C_0) + (C_0 + C_1)]}{2 \cdot (t_1 - t_0 / T)}$$

where C_0 is the baseline GH concentration at time $t = 0$, the beginning of the secretory episode. C_1 is the peak GH level, achieved during this secretory episode at time t_1

$$T = \frac{t_{1/2}}{\ln 2}$$

The GH secreted per peak is the amount necessary to obtain the peak serum concentration $(C_1 - C_0) \cdot V$ added to the amount eliminated during this period. The latter is $\text{MCR} \cdot \text{AUC}$ or $(\ln 2 / t_{1/2}) \cdot V \cdot (t_1 - t_0) \cdot (C_0 + C_1)$. The total 24-hour secretion is the sum of all peak secretions, assuming no basal release.

A1.3 Deconvolution model of Veldhuis, Carlson and Johnson (DECONV) A 24-hour GH profile is the result of secretion of GH by the pituitary gland and concurrent elimination processes in the body.

The concomitant effects of these two processes can be described by a convolution integral. The reverse, going back from the result to the contributing process, is called deconvolution. In its simplest form, convolution is described by the following equation:

$C(t) = S(t)E(t-z)dz$ where $C(t)$ is the concentration GH at time t , $S(t)$ is the secretion function at time (t) , and $E(t-z)$ is the elimination function, which describes the metabolic removal that took place over the time interval $(t-z)$. Veldhuis (1988a,1992) assumes that GH secretion from the pituitary gland takes place as a discrete finite series of molecular bursts of GH release, each of which comprises a Gaussian-shaped pulse of GH secretion with non-zero amplitude. Such a pulse is completely described by its location in time, amplitude and half-duration. To describe the elimination processes, a first-order decay function is used. One of the parameters, $t_{1/2}$, is estimated individually, which is an important feature of this model. All the unknown parameters such as $t_{1/2}$, HD, the amplitudes of the pulses and the positions of the pulses are estimated by an iterative non-linear least-squares parameter estimation algorithm. During this process, the dose-dependent standard errors associated with each sample mean are used in the inverse weighting function. Total hormone secretion per day is calculated as the sum of the masses of hormone secreted (in mU/ml distribution volume) across all the identified secretory bursts. The mass of hormone released per burst equals the square root of 2π times the product of the amplitude of the peak and its SD (SD of the secretory pulse, which equals $HD/2.354$).

A1.4 Deconvolution Model of Johnson, Lassiter and Veldhuis (PULSE)

The difference between this model and that of Hindmarsh, 1990, is the independence of the shape of the secretory pulse and the need to supply a fixed $t_{1/2}$ for the hormone of interest. For the GH elimination process, a twophasic model is used with a 1st component $t_{1/2}$ of 3.5min, a 2nd of 21min and the relative contribution of the slow component to total elimination of 0.63, based on the observations by Faria (1989). The combined criteria of a significant rate of change of secretion (1st derivative) and a nonzero peak secretory rate identify significant hormone secretory peaks. Statistical confidence intervals for these two measures (sample secretory rates and their 1st derivatives) can be propagated by non-linear methods (Veldhuis 1990,1992).

APPENDIX 2.0

PROCEDURES 1: METHODS and EXCLUSIONS

A2.1 Practical procedures

The ethical approval is discussed in Chapter 2 and information sheet provided (Information: A1).

(i) Cannulation and blood sampling

The double lumen catheter was inserted into a main vein at the wrist of the left arm and the second cannula into the antecubital vein of the right arm (Figure A). Continuous integrated blood samples were taken by Watson Marlowe 101U peristaltic pump (Figure B, Watson Marlowe Ltd. Marlborough, Buckinghamshire). The forearm bearing this cannula was maintained in a heated box throughout the morning study or overnight to arterialize venous blood (McGuire 1976).

The blood was prevented from clotting by the use of a double lumen cannula. This was made from a standard venflon (21 gauge, Viggo AB Helsingborg, Sweden) with the valve removed (using a number 7 stainless steel screw). The insert was constructed by using epoxy resin glue and fixing fine bore portex tubing (Portex Ltd. Highes Kent, UK) through a metal needle shaft, itself inserted through a luer cap. The equipment was gas sterilised with ethylene oxide. Heparin 6250 units per ml was infused through the outer lumen of the double cannula at 100 of the rate of blood withdrawal giving a final concentration of heparin in the blood of 62 units per ml. The countercurrent heparin infusion to the inner tube of the blood withdrawing needle was achieved using a hydraulic reduction system. The peristaltic pump had a separate pipe for distilled water, which was pumped up at the same rate as that of the withdrawal of blood: this distilled water was directed into a 100ml capacity glass syringe. The barrel of the 100ml syringe was connected back to back to the valve of a 1ml syringe containing the heparin. The ratio of the two

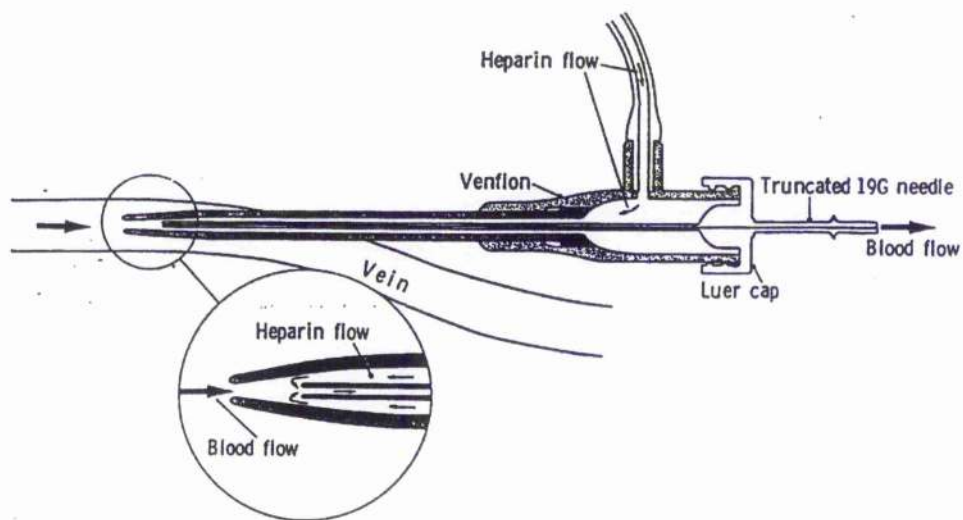


Figure A The double-lumen blood-sampling needle

This consists of a 21G venflon (Viggo,UK) with a Portex tube central lumen fixed to a luer-lock cap using epoxy-resin glue. The inset shows how blood is heparinised within 2mm of the tip of the cannula.

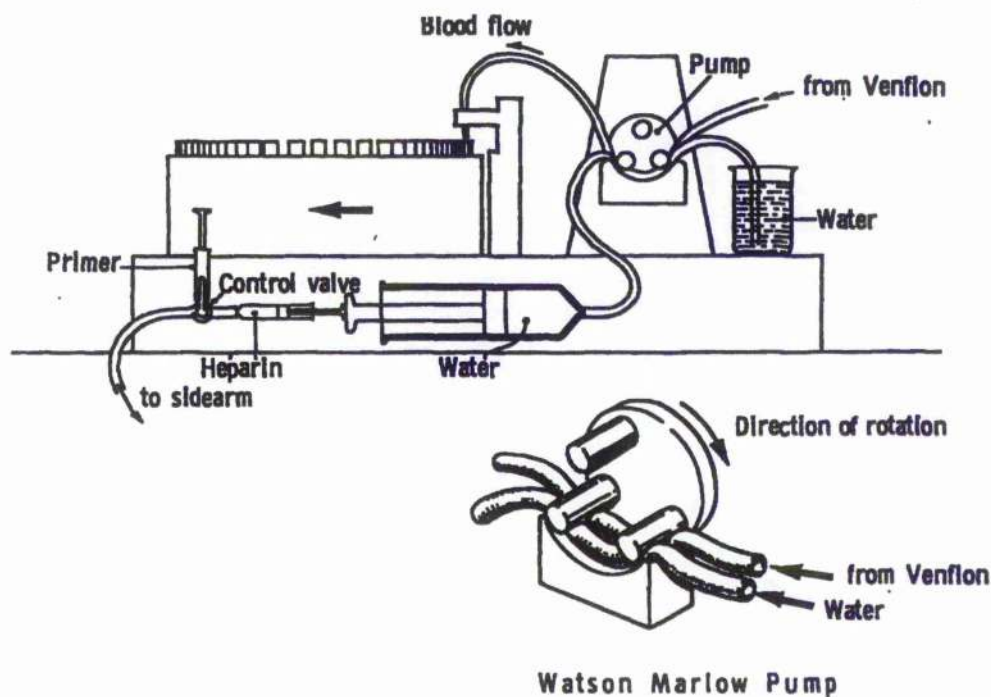


Figure B The continuous blood withdrawal system.

Blood from the patient is taken through a rotary pump to a fraction collector. The first two drops of blood pass into a removable cap on the collecting tubes and is used for glucose analysis. Water is pumped simultaneously with the blood (lower inset) to a hydraulic system consisting of a 100ml glass syringe 'back to back' with a disposable 1ml syringe containing heparin. Thus retrograde heparin can be infused at 1/100 of the rate of withdrawal using a hydraulic ram system.

Investigators :

Dr. D.B. Dunger, Consultant Paediatrician.
Dr. B.R. Pal, Research fellow, Department of Paediatrics, John
Radcliffe Hospital. Oxford 64711, ext. 7799

Dear Dr.

We have been studying growth hormone production in young people with insulin dependent diabetes mellitus.

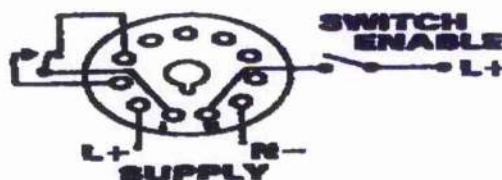
We have shown that adolescents with diabetes have higher levels of growth hormone in the blood compared to normal adolescents. We know that the higher level of Growth Hormone may contribute to problems in glucose control and interfere with the action of insulin as well as having a role in the development of other later complications of diabetes such as eye disease.

We are starting a study to look at the effects that growth hormone has on metabolism and your patient has agreed to join in the study.

The study involves three admissions to one of the children's wards (4B if possible), each overnight. Your patient would arrive around 4.30 pm and have their evening meal in hospital with short acting insulin. On all occasions a cannula would be placed into two arm veins. A drip containing somatostatin infusion to suppress endogenous growth hormone production overnight would then be started through one of the cannulae. During the night exogenous growth hormone/saline would be given through the same cannula at intervals while the patient is asleep. Small volumes of blood would then be taken continuously from the other cannula to measure levels of growth hormone and glucose every 15 mins and insulin, glucose and other metabolites and growth factors in the blood hourly overnight. The study would end at 8.00 am the next day and your patient would be given breakfast with his/her usual insulin and would be able to go to school or work as usual. Your patient will require some minor changes in his/her insulin dose for a day or so beforehand, which will be explained in writing to them. During all the studies, we would give insulin/glucose to try to keep his/her blood glucose levels absolutely stable throughout the night.

None of the substances we would be using has any side-effects in the doses used, and apart from a day or so before the study nights, we would not be changing the usual insulin treatment. We hope that the results will help our understanding of the way that growth hormone levels affects control in diabetes, and may eventually help with some treatment options.

If you would like any further information, or if you do not think your patient should be included in this study, please do not hesitate to contact Dr. Dunger or Dr. Pal at this hospital.



CONNECTIONS (BOTTOM VIEW)

Note: pins 5 and 7 are used internally, do not connect to these.

48 x 48 DIN case
D.70 (body)
panel cut out
48 x 48

A multi-functional digital timer in a 48 DIN housing, suitable for front panel or 11-pin base mounting. The front panel LCD may be set to display elapsed time (count up) or time to elapse (count down), this setting is indicated by a 'Λ' or 'V' symbol on the display. The timing units (HRS, MIN, or SEC), function symbol and output relay status are also clearly displayed. Other features include:

- **Supply voltage 20-240 V a.c. 20-272 V d.c.** nominal, permitting use with any supply voltage within this range
- **Silver cadmium oxide contacts, S.P.D.T. rated** at 7 A, 240 V a.c./30 V d.c. (resistive). 5 A, 240 V a.c. ($\cos \phi = 0.7$)
- **Six timer functions;** on delay, on pulse, delayed pulse ($t = 250$ ms), asymmetrical recycler with pause or pulse start and off delay (constant supply). For operation see 'timer functions' at the end of timers / time switches section.
- **Time ranges:** 0-10-9-99 and 00-1-99-9 sec
0-01-9-99 and 00-1-99-9 min
0-01-9-99 and 00-1-99-9 hrs
- **Internal nickel cadmium rechargeable battery** ensures retention of selected function and other stored settings, in the event of a power failure, for up to 6 months
- **Enable input** which may be used in any operational mode to trigger timing instead of by supply application

Programming is simply accomplished using the two buttons on the timer face. Full instructions are supplied.

technical specification

Supply voltage	18-264 V a.c. 47 to 440 Hz
(Max/Min limits)	18-300 V d.c.
Time setting limits	100 ms to 99-9 hours
Setting accuracy	± 20 ms or $\pm 0.5\%$ of set time whichever is greater
Repeat accuracy	$\pm 0.3\%$ of set time
Mechanical life	1×10^7 operations
Electrical life	2×10^5 operations at rated load
Operating temperature	-10°C to $+60^\circ\text{C}$
Storage temperature	-20°C to $+85^\circ\text{C}$

syringes allows 1ml heparin to be infused for each 100 mls of distilled water pump.

Sampling intervals are given in the respective Chapters. 15minute blood glucose measurements at the bedside were required to maintain adequate clamp conditions. 15minute GH levels were needed to obtain overnight GH pulse profile data but more frequent sampling for bolus half-life data reported in section 2.0.5. The facilities of a digital timer (**Information: A2**) was used to administer GH for GH signal studies.

A2.2 Comparative subject groups for half-life assessment

The subjects attending for GH clearance study (section 2.0.4) allowed calculation of bolus GH clearance. These results were compared to other GH clearance data.

(i) Subjects for half-life GH data after prolonged infusion

GH data was obtained from subjects attending for the GH signal study. A subset of subjects attending for 60minute pulse infusion (HJKLMP), median age 16.5years (range 13-27.9) median weight was 65.8kg (range 44-80.8), the median duration of the disease was 7.5 years (range 4 to 18y); daily insulin dosage was 54 units (30-74), 0.8 u/kg per day (range 0.6-1.1).

A different subset attending for the GH continuous infusion study (JLMNPQ) allowed estimation of steady-state GH (equilibrium), their age 20.5 years (range 14 to 27.9y), median weight 70.5 kg (52-80.8) median duration of diabetes was 9.2 years (range 6.6-18) and daily insulin dosage was 0.9 u/kg per day (range 0.65 - 1.08).

The results of prolonged GH infusion and effect on half-life are discussed in Chapter 3.

(ii) **Historical Control data**

Hindmarsh et al 1989, 1990 (Somatostatin suppression) (extracted from original manuscript)

Six male subjects (median age 19 years, range 17-45) who had been previously studied by Hindmarsh, (1989) was used as control group for bolus GH. Details of 10 normal subjects (age 18-23years) who had undertaken a study for assessment of half-life with different GH exposures (15,30,60 and 180minutes) by (Hindmarsh 1990) are provided in Table 2.1b. One of the control subjects was GH deficient after cranial irradiation; the remainders were normal healthy adult volunteers. These data have been previously reported and the authors used the similar protocol for half-life studies.

Bolus: Following an overnight fast, an indwelling intravenous cannula was inserted in the right forearm for the somatostatin infusion and for the administration of the bolus injection of exogenous GH. A second cannula was placed in the left forearm to obtain the blood samples. An infusion of somatostatin 1-14 ($50\mu\text{g}/\text{m}^2/\text{h}$) was commenced at 09.00h and samples for estimation of serum GH concentration were taken at 15minute intervals for one hour. At 10.00h an intravenous bolus of biosynthetic human GH was administered in a dose of either 50 or 500 mU. Blood was drawn at 1minute intervals for 30mins, at 5minute intervals for the following 30minutes and at 10minute intervals for a final 30minutes. Samples were spun separated and stored at -20°C prior to measurement.

Shah et al 1999 (Octreotide suppression) (Taken from manuscript)

Recent GH data from Shah (1999) was utilised to define the serum GH concentration-decay curves for GH half-life data in healthy adult subjects (controls). Nineteen adult volunteers (13male, 6female) in good health with normal renal, metabolic and endocrine function (Table 2.1b). Bolus (6 male and 6 female) and GH infusion (7 male and 6 female) studies were performed and these data have been recently reported to assess the effects of sex steroid hormone milieu, gender or menstrual cycle.

Bolus: Subjects were pre-treated with octreotide ($1\mu\text{g}/\text{kg}$) infused continuously iv over 1 hour beginning at 07.00h (fasting), 1 hour before GH injection and repeated every 5 hours during study period (Schaefer 1996). One hour after octreotide, each subject received each of three randomly ordered doses ($1, 2$ or $4\mu\text{g}/\text{kg}$) of human recombinant GH by iv over one minute and consecutive injections were carried out 4 hours apart. Before the bolus GH injection and thereafter, blood was samples every 5minutes for 30minutes and then every 30minutes for 3 hours to define the serum GH concentration-curves.

Continuous infusion: GH infusions were preceded by iv octreotide suppression (as described in bolus study) and studies were carried out after overnight fast. After the bolus GH administration, constant infusions of GH were commenced for 240 minutes at rates of $0.5, 1.5$ and $4.5\mu\text{g}/\text{kg}\cdot\text{min}$. Each infusion dose was stopped for 120minutes before starting the next infusion dose. Blood was sampled every 10minutes for 4 hours during the infusions.

The control groups (Hindmarsh, Shah) used different GH assays to that used in this Thesis. These were compared: A high correlation coefficient between the three immunoradiometric assays (NETRIA, Hybritech, Nichols) was established (NETRIA-Nichols: $r = 0.97$, $p < 0.001$; NETRIA-Hybritech $r = 0.98$, $p < 0.0001$). The GH half-life by mono-exponential analysis obtained by NETRIA, Hybritech and Nichols kit were not significantly different: $13.6, 13.7$ and 13.8min respectively.

A2.3 Subject Exclusion

GH Clearance study

Subject A, who was the first subject studied for half-life morning studies, was excluded as practical problems required readjusting.

Subject G did not attend for third morning (family commitments)

GH signal study

Subject H(1-3) had unusual GH profile during one study night suggesting infusion pump/clock timing malfunction and although she attended for 3

studies, metabolic data was disregarded but one night GH data was utilised for half-life studies.

Subjects who developed hypoglycaemia BG <2 mmol/l during use of variable insulin clamp with somatostatin infusion included

Subject K3 and P1 (came back for a P4 night).

Subject refusal

Subject N3 did not attend for third occasion as he refused to be a research subject.

A2.4 Practical Problems and patient discomfort

Overnight metabolic studies using SRIH and glucagon infusions induced nausea and vomiting for 1-6 hours in 5 patients (total of 9(36%) study nights) after stopping infusions at 0800h; two patients developed keto-acidosis because of persistent vomiting, one of these was found to have Rotavirus ultimately. Both glucagon and SRIH decrease gastric motility and cause nausea and vomiting and it was difficult therefore to find the exact cause particularly as other factors such as morning nausea and one patient had clearly identifiable vomiting initiated after removal of intravenous cannula (vasovagal). However, being altruistic adolescents, all but one volunteer, returned to complete all profile nights and for this I am truly grateful.

APPENDIX 3.0

INFUSIONS AND ASSAYS

A3.1 Infusions

(i) *Recombinant human Growth Hormone (r-hGH)*

Somatotropin is the internationally approved name for recombinant DNA-derived 22k-Da human GH.

GH stability - Buffer Solution (control) and GH infusion

Stability of GH as an infusion must be achieved before use in research studies. Advice was obtained from Kabi Pharmaceuticals (Stockholm) regarding the constitution of Buffer solution.

The Buffer solution was made up as follows: *Aminoacetic acid (glycine)* 24mg/ml; *Sodium dehydrogen phosphate(with 12 molecules water)* 0.64904 mg/ml; *monosodium dihydrogen phosphate (with 1 molecule water)* 0.30184 mg/ml.

The GH infusions are described in 2.1.6

(ii) **Insulin**

Insulin infusion was achieved through a separate cannula inserted in the antecubital fossa. The insulin infusion rate was made up to a correction of 0.04 units per ml by adding 20 units of human actrapid insulin (Novo Laboratories) and 2 mls of the subjects blood to 48 mls of 0.9% saline or more dilute at 0.01 units per ml by adding 5 units of human actrapid insulin to the blood. Adjustment of the insulin infusion rate was by syringe pump (Treonic IP3; Vickers pump, Basingstoke, Hants, UK).

(iii) **Somatostatin infusion (Chapter 2 and 5,6)**

During morning studies, a bolus dose of 50µg SRIH was given followed by infusion at 50µg /m²/hour over 270minutes (section 2.1.4).

The overnight Somatostatin infusion (30 mcg/ml) was made by adding 1500mcg of a 3mg vial of somatostatin 1-14 (Stilamin, Serono, S.p.A, Rome, Italy) dissolved in 2ml water, to 49ml 0.9% saline and infused at a rate of 50-100 mcg/m²/h between 19.00-08.00h by a similar Vickers syringe pump. The solution was changed after 6 hours.

(iv) Glucagon infusion (Chapter 6)

The glucagon infusion (1mcg/ml) was 0.5mg of 1mg vial of glucagon (Novo Industri, Bagsvaerd, Denmark) in 10ml 0.9% saline (50mcg/ml) then adding 1ml of this to 49ml 0.9% saline. The same solution and glucagon was used throughout the study and the glucagon was infused through a Vickers syringe pump at a rate of 1ng/kg/min between 19.00-08.00h.

A3.2 Assays

(i) GH

Samples for GH assay were kept at room temperature until the profile was complete and then were spun, separated and the plasma was frozen and stored at -20°C until assay. Plasma GH concentrations were measured by immunoradiometric assay (Netria, St Bartholomew's Hospital, London) using an international reference standard 80/505 and all the samples from subject's overnight profile were analysed in the same batch.

It has been assayed for 30 years in serum or plasma and most immunoassayists would not consider GH heterogeneity to be a major factor affecting the validity or clinical usefulness of such assays.

Comparison of GH assays: Plasma GH was measured by an immunoradiometric assay (Netria, St Bartholomew Hospital, London) using the international reference standard 80/505, and all samples from each patient were analysed together in batch. Sensitivity of the GH assay was 0.3 mU/l (0.15 micrograms per litre); the inter-assay coefficient of variation (CV) at GH concentrations of 3.5, 15.2 and 77.4 mU/L were 9.4%, 7.7% and 10.5%

respectively and intra-assay CV at GH concentration of 2.9, 17.7 and 69.4 mU/L were 8.0%, 4.9% and 3.4% respectively. Samples from two patients (B,E) were also measured with two additional immunoradiometric assays: the Tandems-R immunometric kit (Hybritec, Europe) and the Nichols Institute Diagnostic (St. Juan, Capistrano, CA).

The Hybritec IRMA may not detect 20K GH isoforms, whereas the Nicholls kit (SanJuan/Capistrano, CA) may measure the 20K and the 22K GH forms. In addition the Hybritec kit is highly specific for monomeric GH; the inter assay co-efficient variation for the Hybritec kit was 10.8% at a serum concentration of 5.8 mU/l (2.9 micrograms per litre). The intra assay co-efficient of variation at serum GH levels of 2.8 mU/l (1.4 micrograms per litre), 6.2 mU/l (3.1 micrograms per litre), 26.4 mU/l (13.2 micrograms per litre) and 90.4 mU/l (45.2 micrograms per litre) were 10.6%, 7.8%, 4.9% and 4.9% respectively. Sensitivity was 0.5 mU/l (0.25 micrograms per litre). Standard was IIS 2443E (NI8) which had been recalibrated to milliunits per litre with the first international reference preparation 66/217 (1 micrograms per litre = 2 milliunits per litre).

Nicholls Kit The intra assay co-efficient at GH concentrations of 3.3, 27 and 65mcg per litre were 8.3%, 4.3%, 3.9%. The inter assay co-efficient of variation was 9.7% at a serum concentration of 3.6 micrograms. This precaution was taken as the GH samples from GH half-life studies performed on control subjects by Hindmarsh 1989,1990 were assayed by the Tandems-R immunometric kit (Hybritec, Europe).

The GH levels are reported in all my studies are plasma GH levels measured from the Netria kit unless otherwise stated.

(ii) Insulin

For measurement of plasma free insulin, 1.0ml whole blood was added immediately to 0.6ml ice-cold 25% polyethylene glycol (PEG, mol. Wt. 6000 Sigma Ltd, Poole, UK). Samples were stored at 0° to 4°C, then centrifuged at

3000 rpm for 35 minutes and separated within 13 hours (Collins 1985). The plasma was stored at -20°C and assayed within 3 months. Assay was by double-antibody radioimmunoassay (Guildhay Antisera Ltd., Guilford, UK) modified by Morgan and Lazarow (Morgan 1965). Interassay coefficients of variation at 12.2 and 47.2 mU/L were 5.5 and 8.6% respectively.

(iii) Ketones

For ketone measurement 1.0ml whole blood was added immediately to 3.0ml of ice-cold 10% perchloric acid (PCA) and mixed. Samples were stored at 0° to 4°C for 13 hours, then spun and the precipitate discarded. The supernatant was neutralised using 2% and 20% potassium hydroxide and 10%PCA. Assays of all three metabolites were standard enzymatic techniques (Williamson 1979). Inter- and intra-assay CVs for the βOHB assay were 8.3% and 2.2%; and for the acetoacetate assay 5.3% and 2.2%, respectively. Lactate inter- and intra-assay CVs were 5.1% and 1.0%, respectively.

Glycerol was assayed from the same sample as ketones and the interassay CV was 3.4%. As only a few subjects' profiles had this analysis, I have omitted this in final analysis.

(v) NEFA

Samples for free fatty acids (NEFA) were kept in EDTA at room temperature until the study was complete then spun, separated and stored at -20°C until analysis. Assay was by enzymatic colorimetric kit (Wako Chemicals & Alpha Laboratories, Eastleigh, Hants, UK). The inter-assay CV at 0.39mmol/L was 4.4% and intra-assay CVs at 0.33, 0.62, 0.99 mmol/L were 2.7%, 1.1% and 1.1%, respectively.

(vi) IGF-1

Serum IGF-1 was measured after acid-ethanol extraction then by double antibody RIA by method (Holly 1988).

APPENDIX 4.0

STATISTICS AND ANALYSIS

Statistics is the science of collecting, summarising, presenting and interpreting data and of using them to test hypotheses. During the last two decades it has assumed an increasingly central role in medical investigations. The reasons are many but may be principally that statistics provides a way of organising information on a wider and more formal basis than relying on the exchange of anecdotes and personal experience; more things are now being quantitatively measured in medicine; there is a great deal of intrinsic variation in most biological processes.

A full listing of the statistical texts that have been referred to in this Thesis are provided in the Bibliography and a summary of the main techniques employed are provided in this appendix.

A4.1 Computing of data

Between 1989-1993, most of the calculations were done on the Oxford University IBM computer with some later usage on the Birmingham University Honeywell DPS-8/7OM mainframe computer. The programmes used were OXSTAT ver 4 and 5 and SLIDEWRITE and the BMDP Biomedical Computer Programs (University of California, Berkley, USA). Post 1993, Microsoft Office programmes performed word-processing, data transfer and analysis and Stats view and Minitab programmes.

Standard formulae were used for the calculation of means, standard deviations (SD) and standard error of the mean (SEM). Student's t-test (paired and unpaired), Analysis of Variance and non-parametric tests for non-normalised data (Mann-Whitney U, Spearman rank correlation) were used as appropriate. The results are plotted graphically by computer programmes updated through Microsoft Office programmes.

A4.2 Normalising data

Group results are given as **mean \pm SEM** unless otherwise stated. Where SD are provided this can be converted to $SEM = SD/\sqrt{N}$, where N is number of subjects in study. Blood glucose and insulin infusion data were normally distributed. Log-transformation normalised the ketone, lipid and plasma free insulin data and therefore parametric statistical techniques have been used on these log-transformed data.

A4.3 Statistical analysis

Between 1989-1994, analysis using OXSTAT (Oxford University) and 1998-2000 the online help facility in Minitab 10.51 was utilised.

(i) Students' *t* test

Student's paired *t*-test was used for comparisons of mean data between overnight profiles and at individual time periods.

The statistical method to use to compare two metabolic profiles has been discussed by Nattrass (1982). The choice is between:

Students' *t* test comparing means at each time point. This may show statistically significant differences at specific time points but not show whether there was an overall difference between the profiles, although if the majority of individual time points were different then the whole profile would probably be different. Also the biological significance of a statistical difference at one or two time points during a 12-hour study night is difficult to assess.

A total mean value for a period studied can be calculated for each patient in a group and these values then compared between groups

(ii) ANOVA, Analysis of Variance (one way and two way)

Analysis of variance (2-way ANOVA) was used to examine changes with time and confirm differences between study nights.

Two-way analysis of variance with metabolite concentration classified by group and time (Armitage, Blackwell Science, UK). It is possible with this method to compare the differences in the variance between two groups removing the variance due to the time the samples were taken. Each sample value is used in this method rather than a derived mean. The significance of the difference between groups was calculated from the variance ratio using F-tables.

(iii) Mann-Whitney test

The Mann-Whitney test was used to compare the GH secretion data between puberty groups as this was not normally distributed. The Mann-Whitney test is commonly regarded as a test of medians where one median is an estimate that of the probability that one variable is less than the other (Altman 1991). However, it is a test of both location and shape. Given two independent samples it tests whether one variable tends to have values higher than the other. However, it is also important to look at distribution differences or spread. I have therefore provided the range of GH secretion for each puberty group. This will provide the features that are most clinically important. The hypothesis that there was no difference between puberty groups can be rejected as both median and spread of the difference is different between each puberty group.

(iii) Regression and Correlation

Regression functions can be either linear or of higher form. By changing the x or y scale non-linear functions are always converted, to linear ones, since the mathematical treatment for this is simpler ($y = a + bx$). The gradient **b** is known as the **regression coefficient** and is the angle between the regression line and the abscissa and can be positive or negative (inclined left or right). The gradient **b** shows how many times y is smaller or greater than x. It is calculated by dividing the co-variance of the product (x.y) by the variance of

the individual values x . This relates to the common regression of y to x , where y is the dependent variable and x the independent variable.

The opposite regression, x to y , gives as a rule a regression line somewhat differently inclined to that of the regression y to x . The measure of the mutual behaviour of these two lines gives the **correlation coefficient** r . This is the geometrical mean of the two regression coefficients. The relation between the regression coefficient b_{xy} and the correlation coefficient r is given by

$$r = b_{xy} \frac{S_x}{S_y}$$

The square of the correlation coefficient r^2 is the measure of certainty and shows to what extent the independent variable x influences the dependent variable. A measure of certainty of 0.5 indicates that 50% of the changes in y can be explained as due to changes in x . There is no actual proof that the connection is a causal one and the result should be regarded as a pointer to the direction in which further investigation would yield direct proof.

(iv) Cross-correlation

Cross-correlation is an iterative technique for establishing whether there are statistically coincident recurring waveforms (of any shape) within a data array (Matthews 1983). One data array is serially correlated against the other with progressive step changes in the time-relationship between the data. The result is dependent both on the relative amplitude of such waves or pulses (e.g. whether large pulses of one array are associated with large pulses of the other) and on the phase relationships between the arrays (whether one data array is time-lagged with respect to the other). It is independent of absolute concentrations of hormone.

(v) Probit Transformation

The probit transformation is useful in all cases where a distribution must be tested for normality or where the parameters of a population must be

determined as exactly as possible from a few values which are known to have originated from a normally distributed population. Continuous summation (from left to right) of the area under the normal distribution and plotting the areas against a linear abscissa yields a *sigmoid curve*. The values of its ordinates correspond to the areas of the normal distribution.

The sigmoid curve may be transformed into a straight line by means of the probit transformation, whereby the frequency percentages (=areas x100) are converted into corresponding deviations of the normal distribution increased by the addition of 5 and these are known as *probits*. Probits may be obtained without the need for calculation from tables (Statistical Tables for Agricultural, Biological and Medical Research, 1953 by RA Fisher and F Yates; published by Oliver and Boyd, Edinburgh).

Optional Calculations

CF = Cumulative frequency

EP = Empirical probits (derived from statistical tables, Fisher & Yates 1953)

PP = Provisional Probit This is obtained from the regression line on probability paper

This calculates mean value and A co-ordinates

A (when $x=0$, y = the minimum PP)

Maximum PP is at maximum GH concentration

The points of intersection at x provide the provisional probits

Values for the GH concentrations are then read off

N = Provisional Probit x cumulative frequency (CF) ratio

WP = working probit is (N + minimum PP) for each class

WC = weighting coefficients is PP x number of samples

(vi) Confidence Intervals

The confidence interval is the range on either side of a sample mean. Alpha (α) is the significance level used to compute the confidence level. The confidence level equals $100 \times (1 - \alpha)\%$ or an α of 0.05 indicates a 95% confidence level. If α is assumed to equal 0.05, the area under the standard normal distribution curve

that equals $(1-\alpha)$, or 95% is ± 1.96 and the confidence interval is therefore calculated as

$$\text{Mean } (x) \pm 1.96 \left(\frac{\sigma (SD)}{\sqrt{N}} \right)$$

A4.4 Missing Data

Missing data were handled as follows. On only 11 sampling occasions was insufficient blood obtained and on a further 9 occasions laboratory problems led to the loss of single measurements. The missing values were replaced by the mean of the previous and subsequent measurements. In all this amounted to 0.3% of the metabolic and hormone data.

Main Statistical References used in this Thesis are provided in Bibliography

APPENDIX 5

INSULIN-VARYING CLAMP

Glucose clamping (DeFronzo 1979), whereby a preselected plasma glucose concentration is maintained in spite of the action of endogenous or exogenous insulin, has become a commonly used physiological tool to examine both insulin sensitivity and beta-cell function. Both hyperglycaemic and euglycaemic clamps have frequently been used and a variety of algorithms have been published (Pacini 1982) which achieve clamping by altering glucose infused. However, all these methods use a fixed mathematical formula as a basis of their calculation, requiring the insertion of several constants that make some basic physiological assumptions about the insulin requirement at the outset. There are also disadvantages to such a system of maintenance of blood glucose concentration as both hyperglycaemic and euglycaemic clamps are often undertaken against a background of high insulin concentration.

These always make some basic physiological assumptions about the insulin requirement at the outset with the possibility of biasing the results. An unbiased method of similarly using an insulin infusion is therefore needed to more accurately measure insulin requirements particularly during adolescence when these requirements may be changing substantially. Insulin-varying glucose clamping is more difficult but has been used previously in the form of a prefixed sliding scale or the Biostator or other algorithm. These cannot be made during puberty when insulin resistance is known to change. In assessing Type 1 diabetes the quantity of insulin required for maintenance of basal state can be more informative clinically, and under such circumstances one wishes to vary insulin rather than glucose.

A5.1 Principle of the programme developed by Matthews et al 1989

Minimal models have been utilised for glucose-clamping (Pacini 1982) and most of these algorithms for clamping are

$$\text{New prediction} = f[\text{pre-existing state}] + f[\text{current data}] + f[\text{time}]$$

The data for each function(f) are discrete and usually based only on one or two readings. The new predictions depend critically on formulae that are preconceptions of the way that the body functions or are derived from mean approximations of glucose change. Some have used arbitrary 'factors' to compensate for changing circumstances during a clamp. The computer programme developed by Matthews 1989, adopts 'experience' rather than preconception as the basis of its prediction. By abandoning a fixed algorithm it is possible to accumulate many data entries into an internal table or array. The data for the internal array are calculated as the change of glucose concentration that has occurred against the insulin infusion rate given which caused the change. There is a time lag of insulin action that is built into the programme. Every new glucose concentration reading is matched with the 20-40min previous insulin-infusion datum and inserted into the array. Duplicate data to either infusion data or rate of change data that pre-exist in the array are pooled using an arithmetic mean and the rank order of infusion data is maintained by sequential sorting. If the infusion rates are within 1% of each other, they are averaged so that the array in the computer does not linearly increase in size throughout the clamp. The initial array is 3 values only and this increases progressively throughout the clamp. From this data it is possible to read directly, or by linear interpolation, the insulin infusion rate that will be required to change the observed glucose concentration to that required. The minimal assumption made is therefore that after accumulating data about cause and effect, one may then use the knowledge to predict an effect from a cause.

A5.2 Use of the Programme

1. Starting assumptions

In practice an initial assumption is required to establish an infusion rate. The basic tenet is that over a short period of time there will be no change of glucose concentration if no insulin is given. The array consists of three points only at this stage. Once the programme is running even this minimal assumption is rapidly abandoned in favour of the accumulated information from successive sampling.

2. Adjustments of array during clamp

The accumulated array of data (rate of change in glucose concentration versus insulin infusion rate) is continuously updated and rationalised by averaging, sorting and deletion of redundant data.

3. Lag phase

Changes in blood glucose do not occur instantaneously with changes in insulin infusion. There are delays in mixing, sampling (dead-space) and assaying the blood glucose concentration. The delay can be found in any system by cross-correlation or simply by checking the time lag (y min) between insulin administration and a change in glucose being measured. This data can be used by the programme that then assumes that the current glucose concentration is consequent on the infusion that was running y min before the reading. Inaccuracies in this estimate are not critical because the averaging and ranking allows for 'noise' in the system.

4. Outliers

Assay points that are outliers (eg an individual poor assay of glucose) cause a response in insulin advice immediately. The data are entered by the computer in the periphery of the array. If the next glucose value is a non-outlier, the

central area of the array is utilised again and the outlier has not interfered with the near clamp-level part of the array. Thus the overall effect of an outlier is small and a noisy assay does not lead to an oscillatory phenomena. There are no continuing perturbations from one outlying result.

5. Flexible options

Sample time interval: Because the array is accumulated in terms of concentration change against infusion rates, it is immaterial, within certain limits, what the blood sampling interval actually is. Nevertheless the system time-characteristics are constrained by the delay in insulin action and in practice 10-15min sampling is the shortest time that can be used. The upper limit is constrained by the fact that too long a sampling interval will not monitor changes adequately, and in practice 30 minutes is probably the longest advisable time between samples. The sample interval can be changed easily at any time.

Reaction speed: The computer prediction for infusion rate can be adjusted to correct the current blood glucose concentration to the requested concentration over a range between 30 and 60 minutes. Slow reaction speeds are inefficient and fast reaction times may cause overshoot. In practice, if one wishes the changes to be approximately exponential towards the requested clamp level, then an achievement of two-thirds of the deviation between successive samples will achieve this. Thus the reaction speed is set within the programme as being 1.5 times the sample interval. The reaction interval can be lengthened to reduce deviations in the infusion rates, or if the blood glucose concentration 'hunts' about a mean.

Changing the clamp level: The clamp level can be changed at any time to allow a change from 'basal' to 'clamp' period.

Disc storage: Data are accumulated on disc every time the data array is updated. Interim and terminal storage and print-outs can be easily requested.

This method is an effective, cheap and unbiased method of quantifying insulin requirement. It has been validated in adolescents with Type 1 (insulin-dependent) diabetes (Matthews 1990) and this system has been shown to be sensitive maintaining a clamp glucose concentration of 5.3 ± 0.2 mmol/L (blood glucose remained above 3.0 mmol/L at all times and no glucose was needed for the correction of hypoglycaemia). Thus the insulin infusion rate is equal to the insulin requirement for the maintenance of euglycaemia.

APPENDIX 6

RAW DATA

A6.1 Illustration of overnight GH (profiles and deconvolution)

A6.2 Probit Tables

A6.3 GH signal: IGF-1 and cortisol

Deconvolution data

Raw data is displayed by puberty group and sex. Each profile shows secretion rate, plasma GH and calculated variable half-life.

Overnight (20.00-08.00h) GH profiles taken at 15 minute intervals were deconvoluted by the method of Hindmarsh 1990. Diabetic subjects have normal insulin regime nights (NR) compared with their own respective individual euglycaemic clamp night. Control (normal healthy puberty-matched) subjects are displayed by sex and puberty group in the following section.

Key

X axis = time in minutes

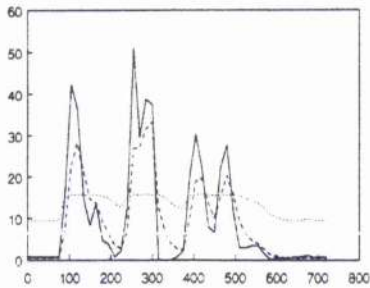
y axis = Plots relate to : GH secretion (mU/min) or Plasma GH (mU/L) or Half-life (mins)

——— Secretion
Rate
----- Plasma GH
..... Calc T.5

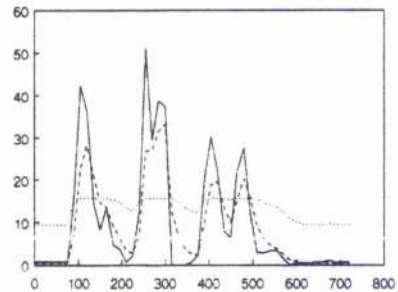
NR normal insulin regime
EC euglycaemic clamp night

Type 1 diabetes

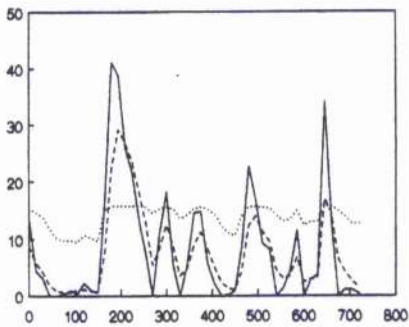
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male puberty 1 study night: NR



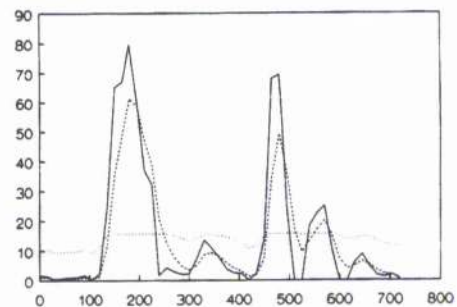
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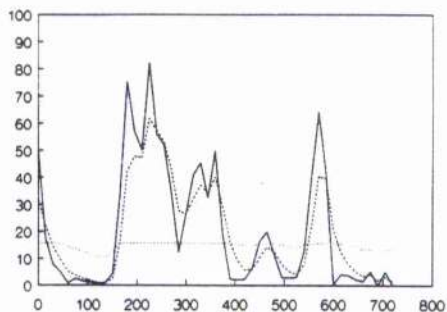
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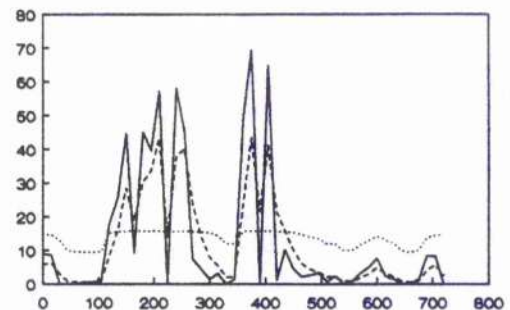
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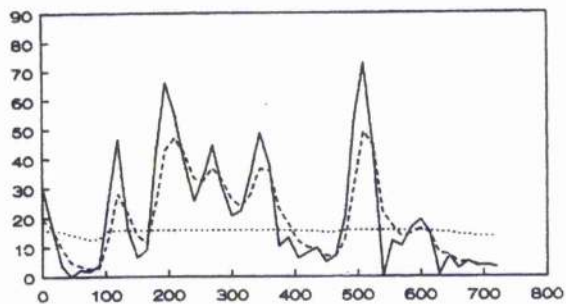
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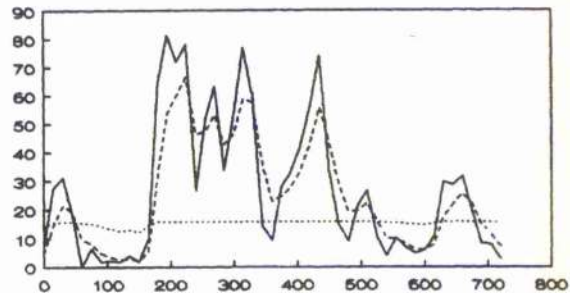
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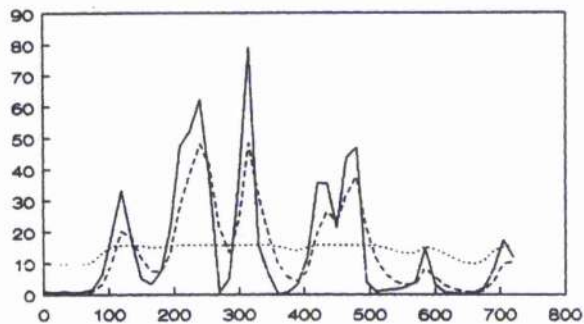
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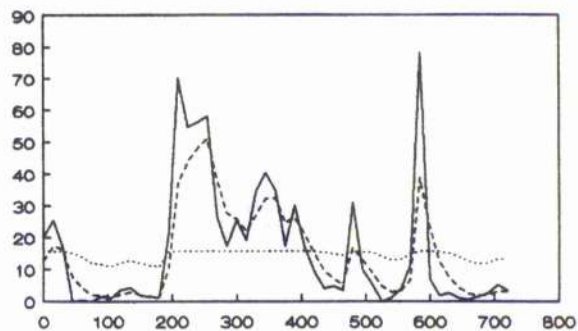
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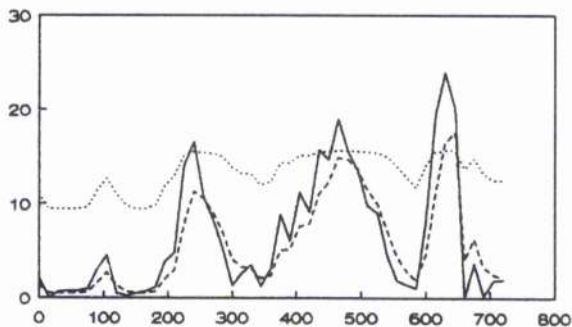
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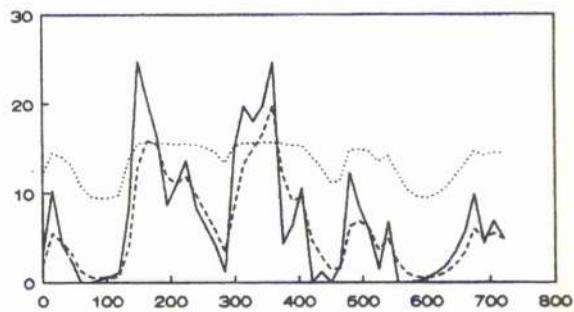
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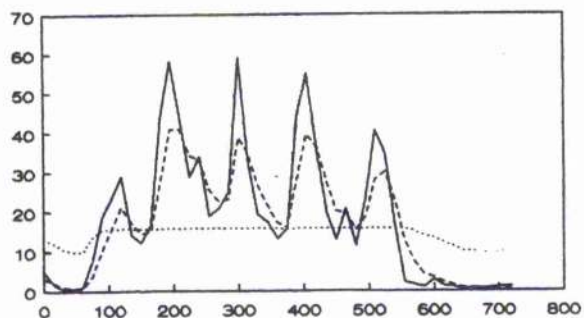
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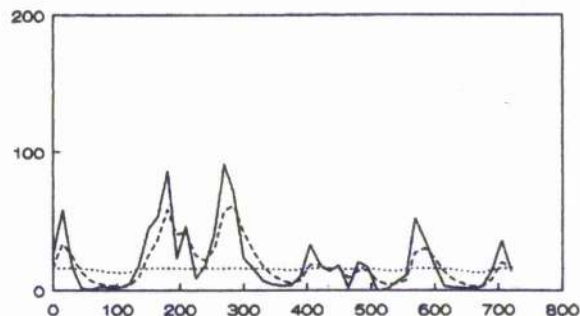
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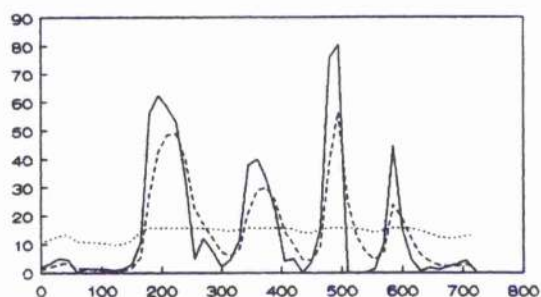
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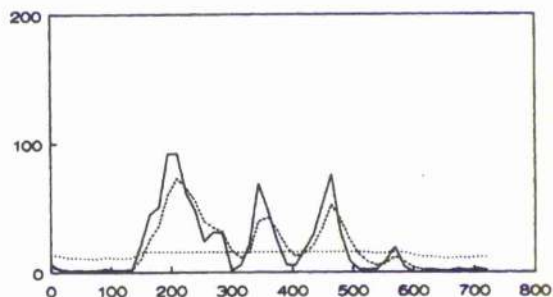
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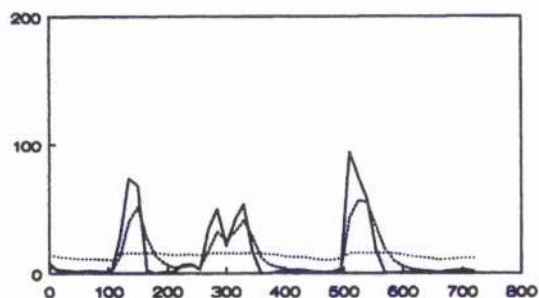
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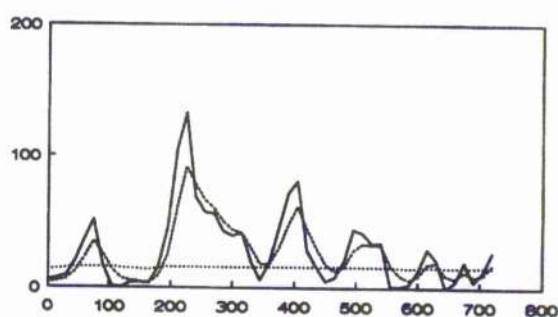
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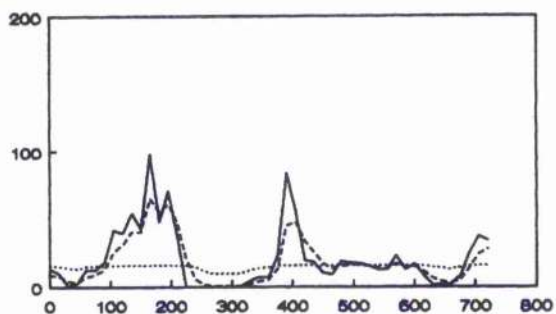
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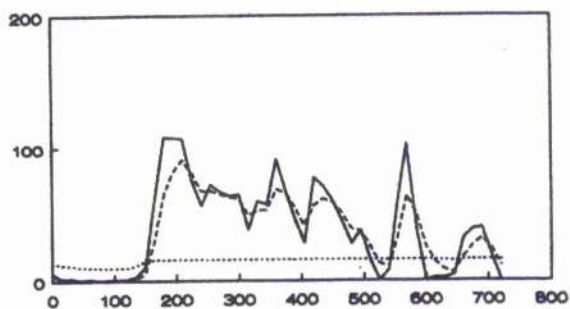
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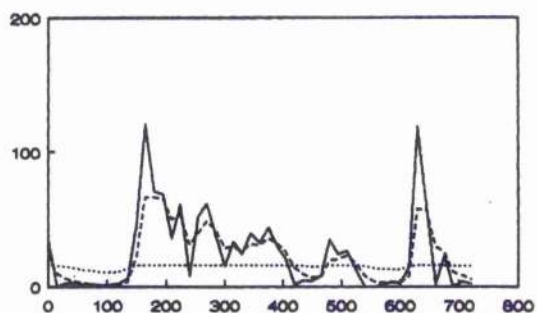
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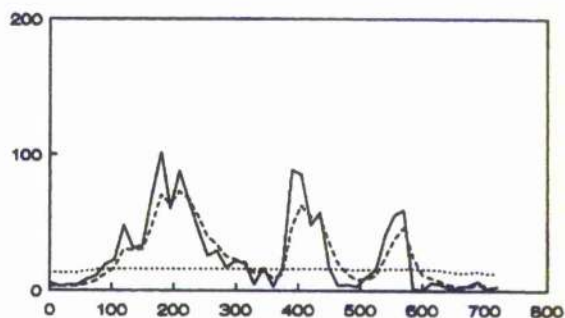
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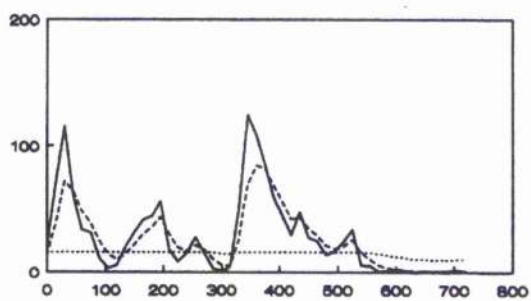
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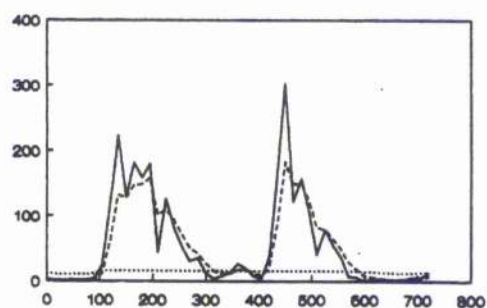
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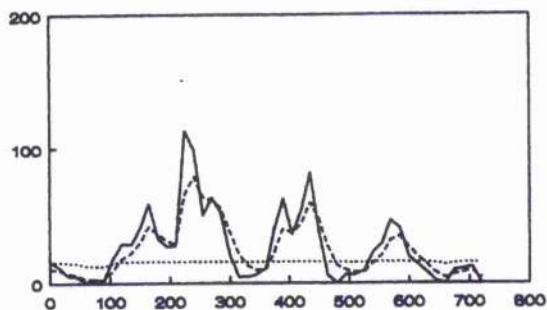
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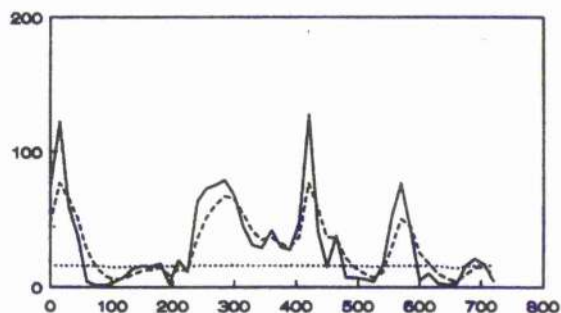
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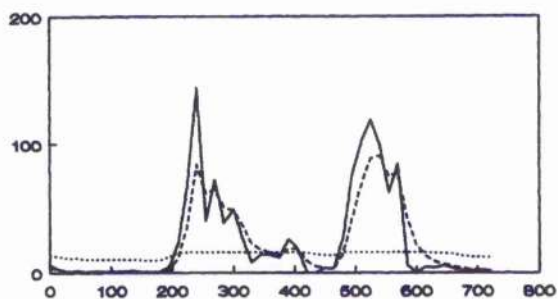
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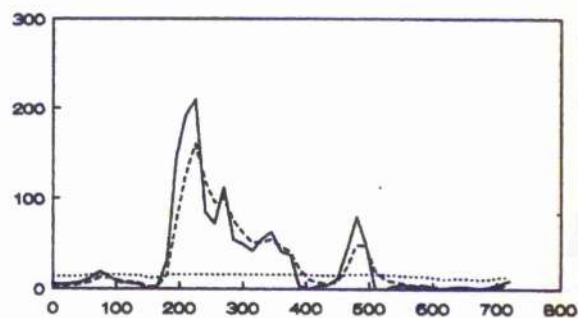
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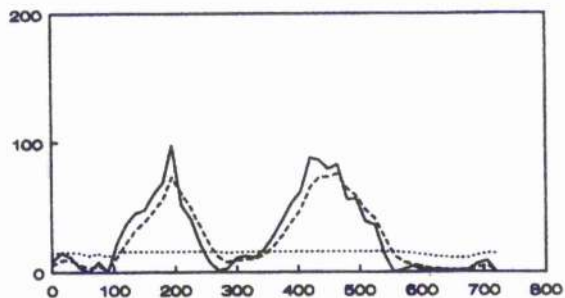
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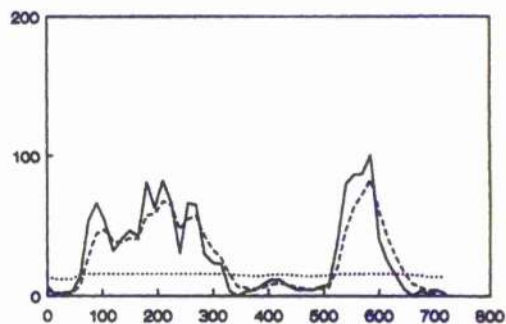
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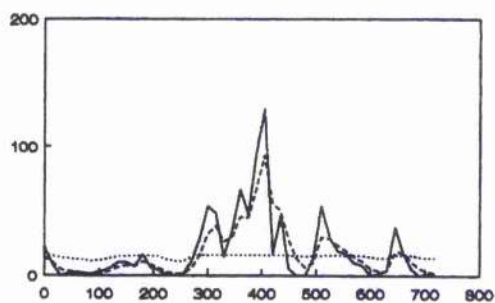
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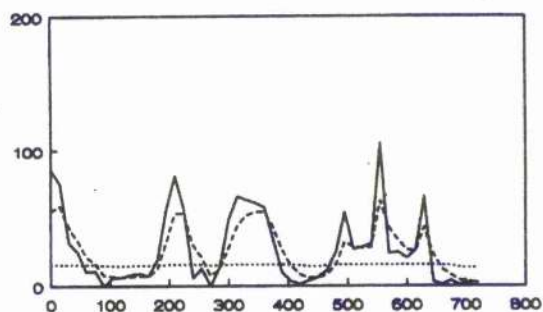
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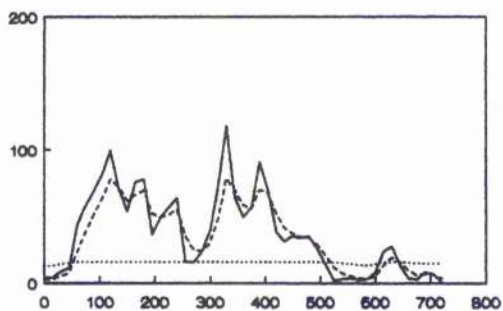
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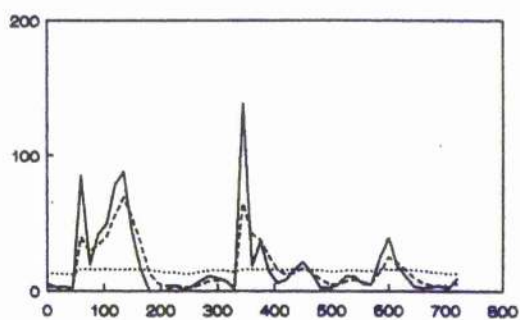
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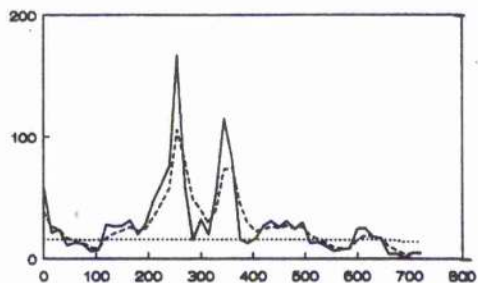
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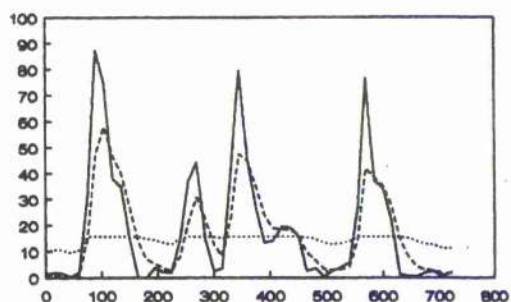
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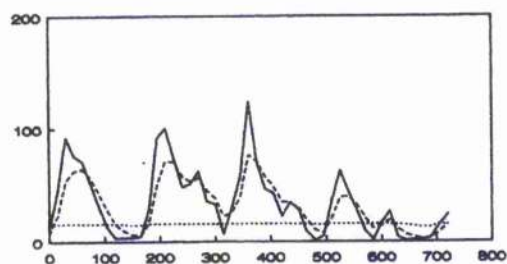
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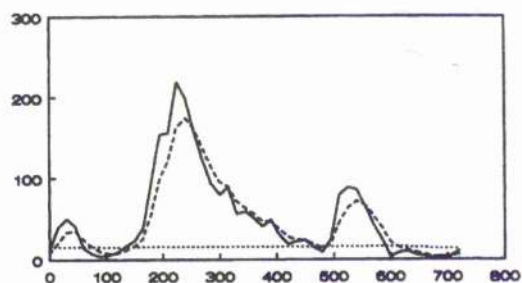
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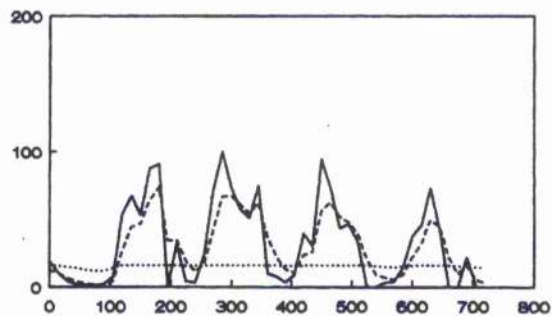
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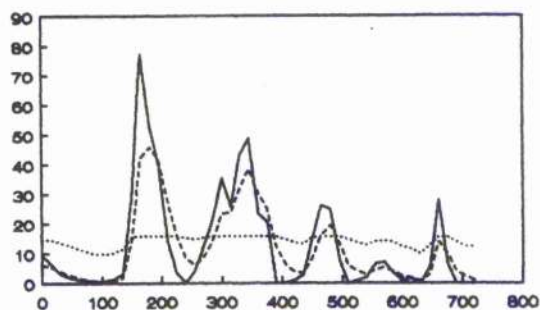
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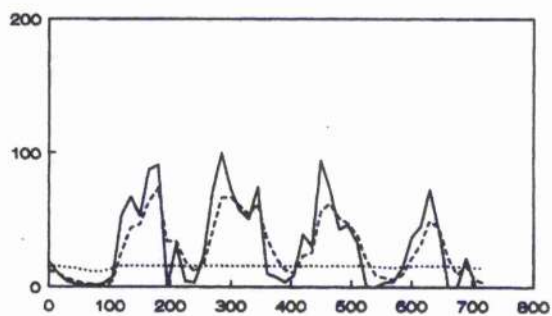
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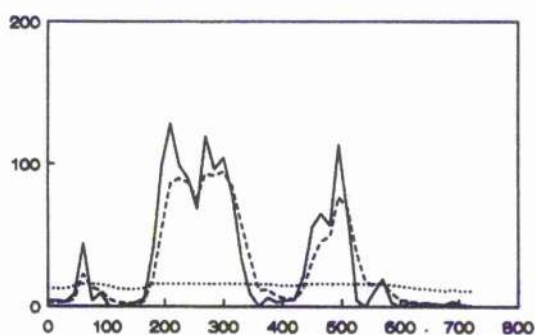
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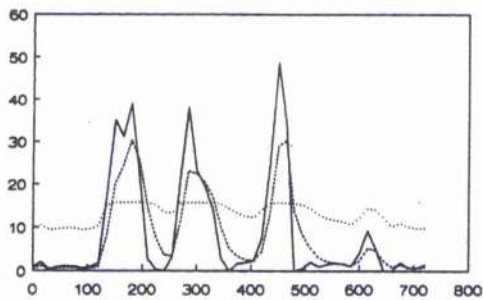


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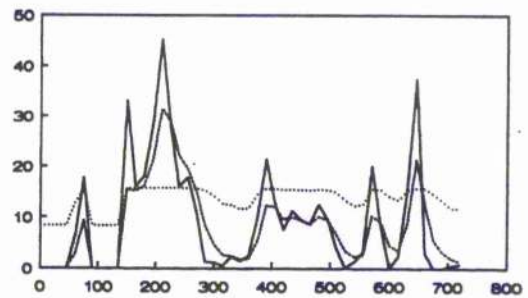


CONTROL DATA

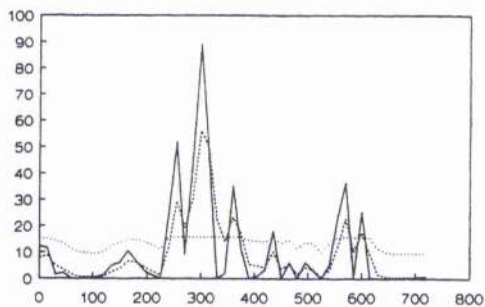
Subject: pm31
male puberty 1 study night: control



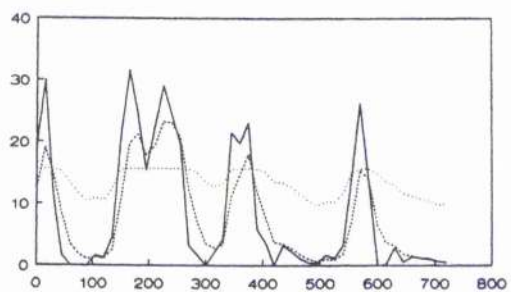
Subject: dc31
male puberty 1 study night: control



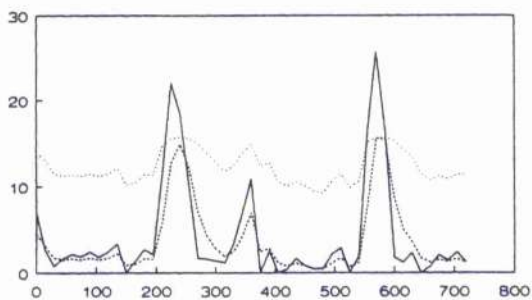
Subject: sw01
male puberty 1 study night: control



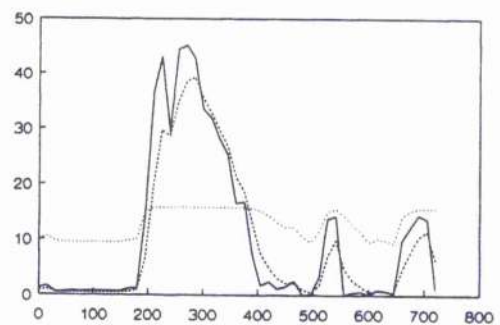
Subject: an34
female puberty 1 study night: control



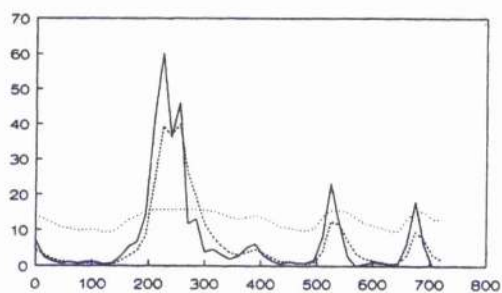
Subject: jg32
female puberty 1 study night: control



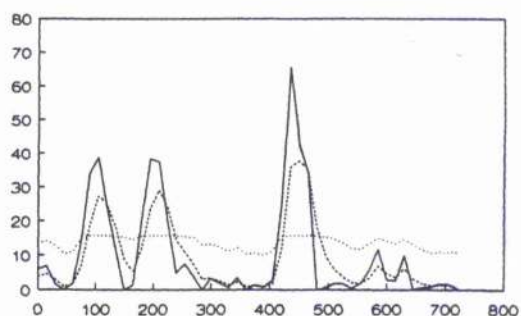
Subject: dm27
female puberty 2 study night: control



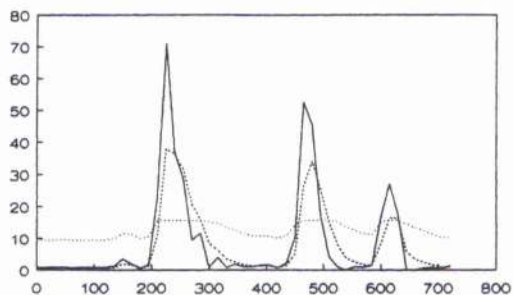
Subject: cp11
female puberty 1 study night: control



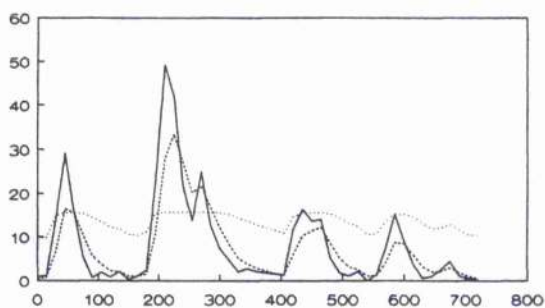
Subject: nt35
male puberty 2 study night: control



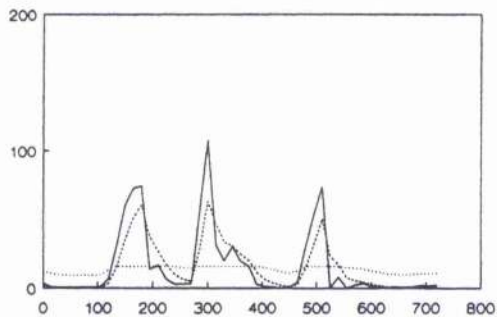
Subject: cg25
male puberty 2 study night: control



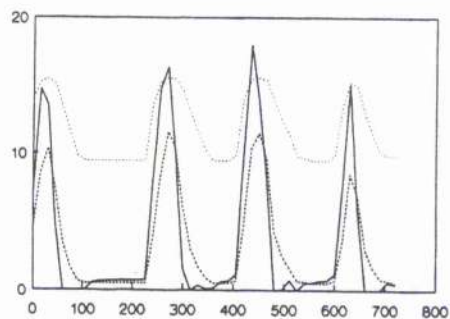
Subject: mb17
male puberty 2 study night: control



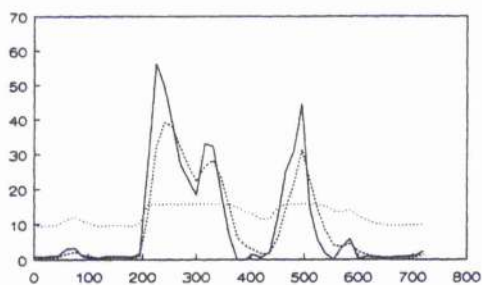
Subject: kk14
male puberty 2 study night: control



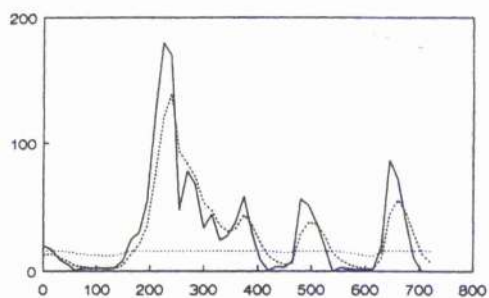
Subject: mb08
male puberty 2 study night: control



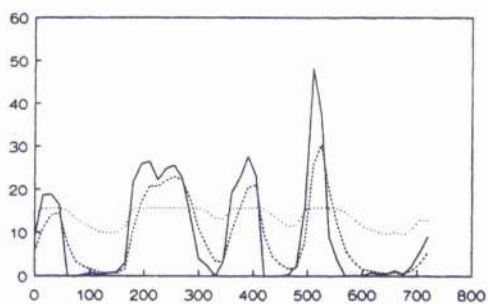
Subject: tg04
male puberty 2 study night: control



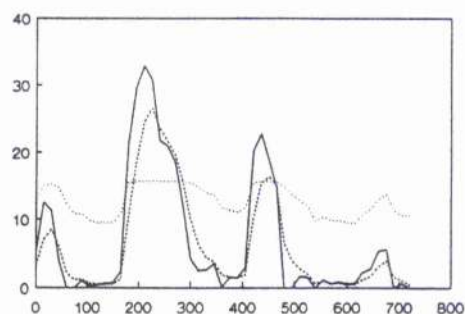
Subject: vr10
female puberty 2 study night: control



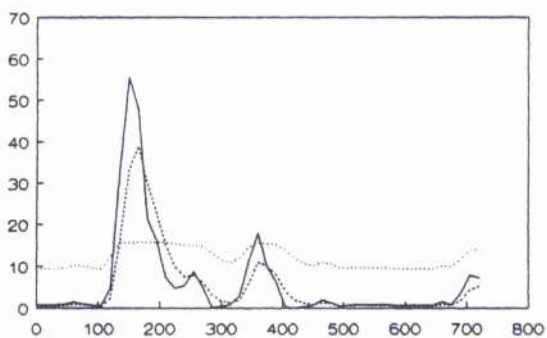
Subject: er03
female puberty 2 study night: control



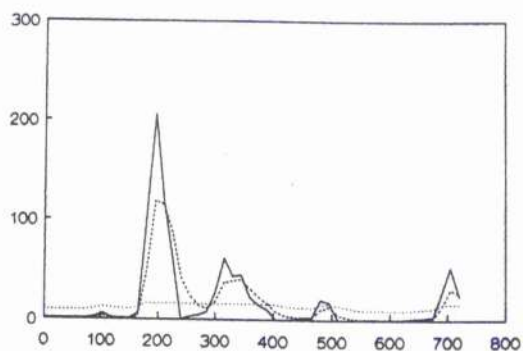
Subject: sd29
male puberty 3 study night: control



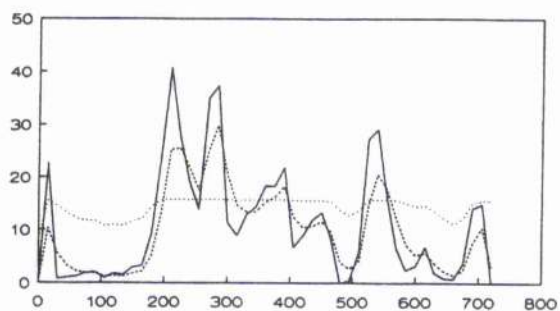
Subject: am15
male puberty 3 study night: control



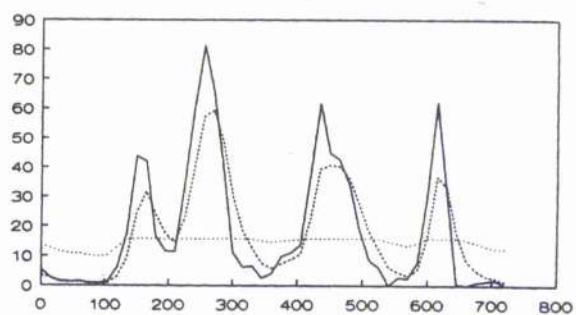
Subject: ik12
female puberty 3 study night: control



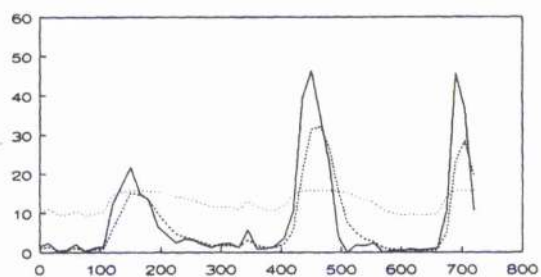
Subject: jc30
male puberty 4 study night: control



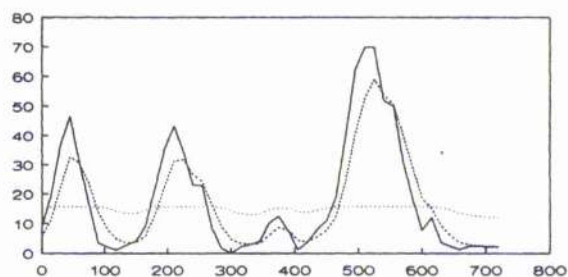
Subject: bw13
male puberty 4 study night: control



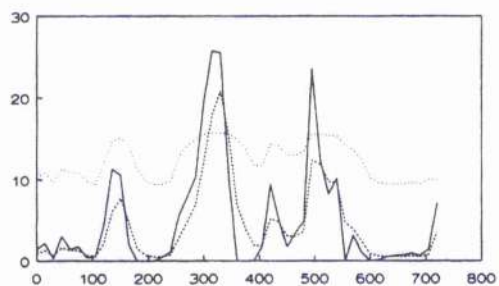
Subject: vm21
female puberty 4 study night: control



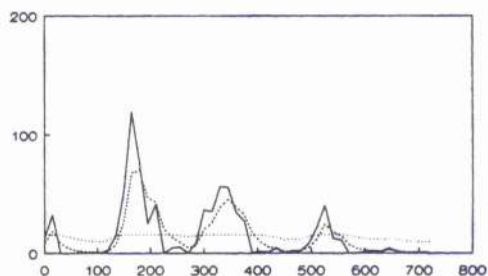
Subject: kh33
female puberty 4 study night: control



Subject: aq05
female puberty 4 study night: control

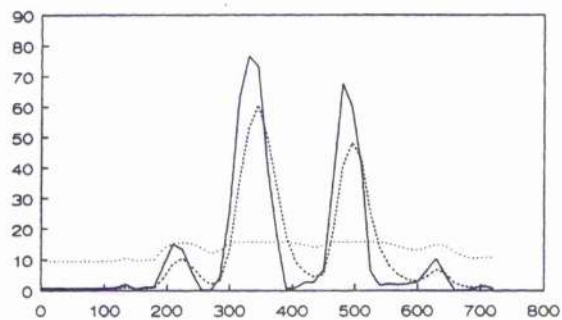


Subject: sc02
male puberty 5 study night: control



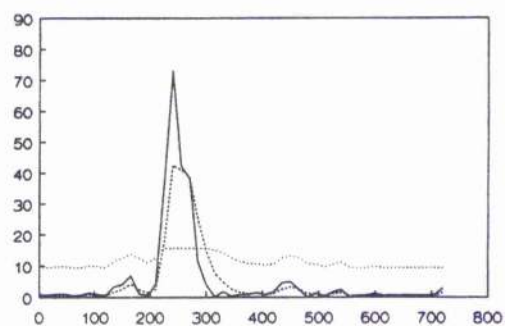
Subject: nk16

male puberty 5 study night: control



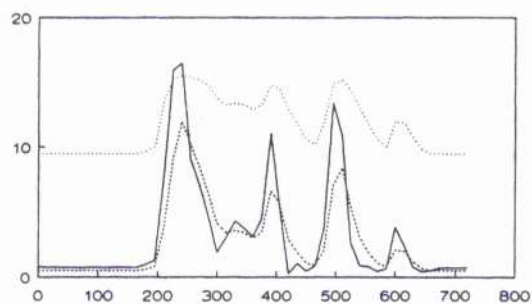
Subject: mc18

male puberty 5 study night: control



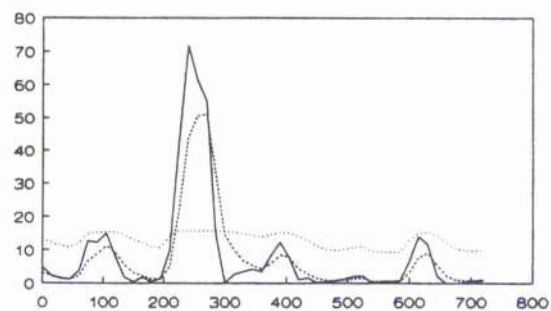
Subject: nb26

male puberty 5 study night: control



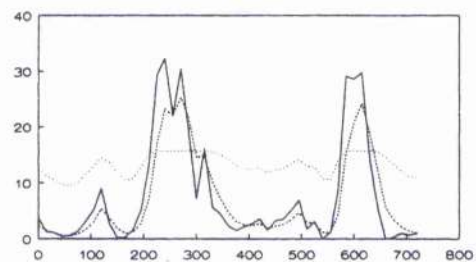
Subject: tr09

female puberty 5 study night: control



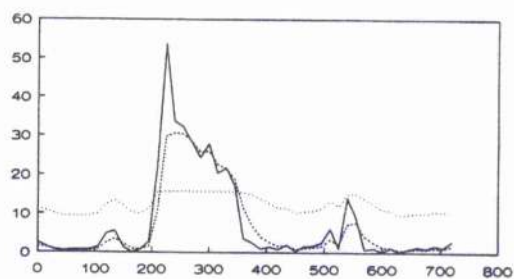
Subject: jm19

female puberty 5 study night: control

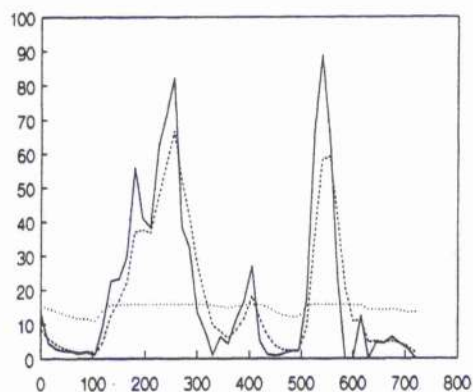


Subject: sm20

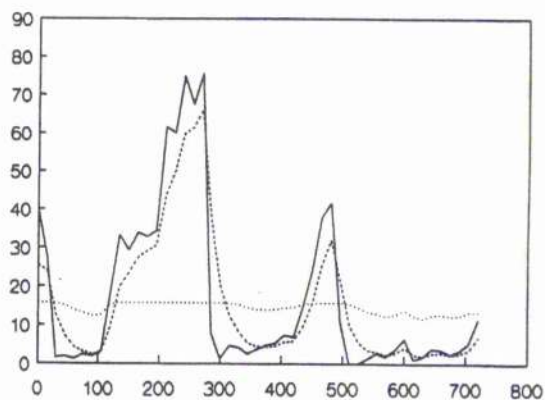
female puberty 5 study night: control



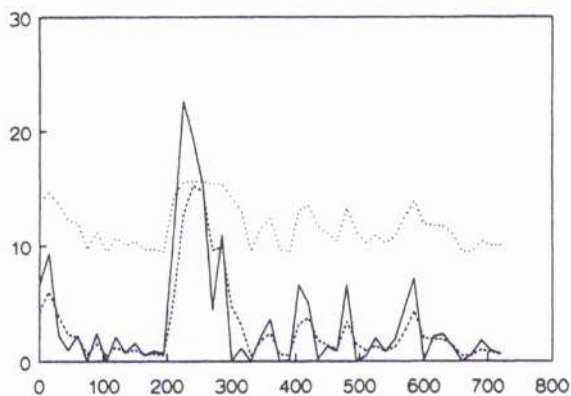
Subject: cs22
female puberty 5 study night: control



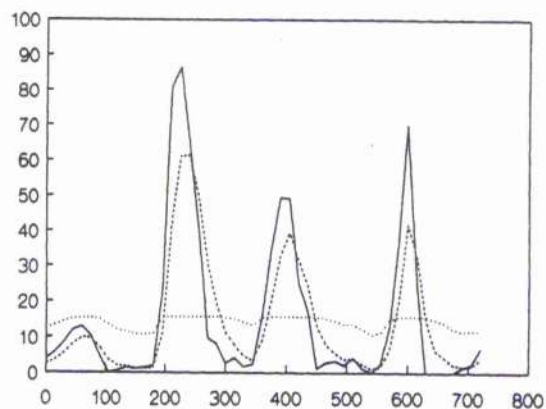
Subject: ci23
female puberty 5 study night: control



Subject: dn24
female puberty 5 study night: control



Subject: tm27
female puberty 5 study night: control



[Transformation of the sigmoid curve into a straight line by transformation of percentages into logits.]

	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	2	3	4	5
	Probit													
0					
1	2.6737	2.6986	2.7235	2.7483	2.7731	2.7979	2.8227	2.8475	2.8723					
2	2.8963	2.9211	2.9459	2.9707	2.9955	3.0203	3.0451	3.0699	3.0947					
3	3.1185	3.1433	3.1681	3.1929	3.2177	3.2425	3.2673	3.2921	3.3169					
4	3.3407	3.3655	3.3903	3.4151	3.4399	3.4647	3.4895	3.5143	3.5391					
5	3.5639	3.5887	3.6135	3.6383	3.6631	3.6879	3.7127	3.7375	3.7623	9	10	27	36	45
6	3.7871	3.8119	3.8367	3.8615	3.8863	3.9111	3.9359	3.9607	3.9855	9	14	24	33	42
7	3.9993	4.0241	4.0489	4.0737	4.0985	4.1233	4.1481	4.1729	4.1977	9	14	24	33	42
8	4.2215	4.2463	4.2711	4.2959	4.3207	4.3455	4.3703	4.3951	4.4199	9	14	24	33	42
9	4.4437	4.4685	4.4933	4.5181	4.5429	4.5677	4.5925	4.6173	4.6421	9	14	24	33	42
10	4.6659	4.6907	4.7155	4.7403	4.7651	4.7899	4.8147	4.8395	4.8643	9	14	24	33	42
11	4.8881	4.9129	4.9377	4.9625	4.9873	5.0121	5.0369	5.0617	5.0865	9	14	24	33	42
12	5.1097	5.1345	5.1593	5.1841	5.2089	5.2337	5.2585	5.2833	5.3081	9	14	24	33	42
13	5.3319	5.3567	5.3815	5.4063	5.4311	5.4559	5.4807	5.5055	5.5303	9	14	24	33	42
14	5.5541	5.5789	5.6037	5.6285	5.6533	5.6781	5.7029	5.7277	5.7525	9	14	24	33	42
15	5.7763	5.8011	5.8259	5.8507	5.8755	5.9003	5.9251	5.9499	5.9747	9	14	24	33	42
16	5.9985	6.0233	6.0481	6.0729	6.0977	6.1225	6.1473	6.1721	6.1969	9	14	24	33	42
17	6.2207	6.2455	6.2703	6.2951	6.3199	6.3447	6.3695	6.3943	6.4191	9	14	24	33	42
18	6.4429	6.4677	6.4925	6.5173	6.5421	6.5669	6.5917	6.6165	6.6413	9	14	24	33	42
19	6.6651	6.6899	6.7147	6.7395	6.7643	6.7891	6.8139	6.8387	6.8635	9	14	24	33	42
20	6.8873	6.9121	6.9369	6.9617	6.9865	7.0113	7.0361	7.0609	7.0857	9	14	24	33	42
21	7.1095	7.1343	7.1591	7.1839	7.2087	7.2335	7.2583	7.2831	7.3079	9	14	24	33	42
22	7.3317	7.3565	7.3813	7.4061	7.4309	7.4557	7.4805	7.5053	7.5301	9	14	24	33	42
23	7.5539	7.5787	7.6035	7.6283	7.6531	7.6779	7.7027	7.7275	7.7523	9	14	24	33	42
24	7.7761	7.8009	7.8257	7.8505	7.8753	7.8991	7.9239	7.9487	7.9735	9	14	24	33	42
25	7.9973	8.0221	8.0469	8.0717	8.0965	8.1213	8.1461	8.1709	8.1957	9	14	24	33	42
26	8.2195	8.2443	8.2691	8.2939	8.3187	8.3435	8.3683	8.3931	8.4179	9	14	24	33	42
27	8.4417	8.466												

* *United States Government Printing Office, Washington, D.C. 20540, 1975. In R. H. Pomeroy and F. W. Wern, published by Croom and Helm, Edin.*

The Normal Distribution — Probit Transformation (continued)

$\Phi^{-1}(p)$	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	2	3	4	5
	Probit														
60	5.2935	5.2999	5.3065	5.3131	5.3197	5.3263	5.3329	5.3395	5.3461	5.3527	3	5	6	10	13
61	5.2999	5.3065	5.3131	5.3197	5.3263	5.3329	5.3395	5.3461	5.3527	5.3593	3	5	6	10	13
62	5.3065	5.3131	5.3197	5.3263	5.3329	5.3395	5.3461	5.3527	5.3593	5.3659	3	5	6	11	13
63	5.3131	5.3197	5.3263	5.3329	5.3395	5.3461	5.3527	5.3593	5.3659	5.3725	3	5	6	11	13
64	5.3197	5.3263	5.3329	5.3395	5.3461	5.3527	5.3593	5.3659	5.3725	5.3791	3	5	6	11	13
65	5.3263	5.3329	5.3395	5.3461	5.3527	5.3593	5.3659	5.3725	5.3791	5.3857	3	5	6	11	13
66	5.3329	5.3395	5.3461	5.3527	5.3593	5.3659	5.3725	5.3791	5.3857	5.3923	3	5	6	11	13
67	5.3395	5.3461	5.3527	5.3593	5.3659	5.3725	5.3791	5.3857	5.3923	5.3989	3	5	6	11	13
68	5.3461	5.3527	5.3593	5.3659	5.3725	5.3791	5.3857	5.3923	5.3989	5.4055	3	5	6	11	13
69	5.3527	5.3593	5.3659	5.3725	5.3791	5.3857	5.3923	5.3989	5.4055	5.4121	3	5	6	11	13
70	5.3593	5.3659	5.3725	5.3791	5.3857	5.3923	5.3989	5.4055	5.4121	5.4187	3	5	6	11	13
71	5.3659	5.3725	5.3791	5.3857	5.3923	5.3989	5.4055	5.4121	5.4187	5.4253	3	5	6	11	13
72	5.3725	5.3791	5.3857	5.3923	5.3989	5.4055	5.4121	5.4187	5.4253	5.4319	3	5	6	11	13
73	5.3791	5.3857	5.3923	5.3989	5.4055	5.4121	5.4187	5.4253	5.4319	5.4385	3	5	6	11	13
74	5.3857	5.3923	5.3989	5.4055	5.4121	5.4187	5.4253	5.4319	5.4385	5.4451	3	5	6	11	13
75	5.3923	5.3989	5.4055	5.4121	5.4187	5.4253	5.4319	5.4385	5.4451	5.4517	3	5	6	11	13
76	5.3989	5.4055	5.4121	5.4187	5.4253	5.4319	5.4385	5.4451	5.4517	5.4583	3	5	6	11	13
77	5.4055	5.4121	5.4187	5.4253	5.4319	5.4385	5.4451	5.4517	5.4583	5.4649	3	5	6	11	13
78	5.4121	5.4187	5.4253	5.4319	5.4385	5.4451	5.4517	5.4583	5.4649	5.4715	3	5	6	11	13
79	5.4187	5.4253	5.4319	5.4385	5.4451	5.4517	5.4583	5.4649	5.4715	5.4781	3	5	6	11	13
80	5.4253	5.4319	5.4385	5.4451	5.4517	5.4583	5.4649	5.4715	5.4781	5.4847	3	5	6	11	13
81	5.4319	5.4385	5.4451	5.4517	5.4583	5.4649	5.4715	5.4781	5.4847	5.4913	3	5	6	11	13
82	5.4385	5.4451	5.4517	5.4583	5.4649	5.4715	5.4781	5.4847	5.4913	5.4979	3	5	6	11	13
83	5.4451	5.4517	5.4583	5.4649	5.4715	5.4781	5.4847	5.4913	5.4979	5.5045	3	5	6	11	13
84	5.4517	5.4583	5.4649	5.4715	5.4781	5.4847	5.4913	5.4979	5.5045	5.5111	3	5	6	11	13
85	5.4583	5.4649	5.4715	5.4781	5.4847	5.4913	5.4979	5.5045	5.5111	5.5177	3	5	6	11	13
86	5.4649	5.4715	5.4781	5.4847	5.4913	5.4979	5.5045	5.5111	5.5177	5.5243	3	5	6	11	13
87	5.4715	5.4781	5.4847	5.4913	5.4979	5.5045	5.5111	5.5177	5.5243	5.5309	3	5	6	11	13
88	5.4781	5.4847	5.4913	5.4979	5.5045	5.5111	5.5177	5.5243	5.5309	5.5375	3	5	6	11	13
89	5.4847	5.4913	5.4979	5.5045	5.5111	5.5177	5.5243	5.5309	5.5375	5.5441	3	5	6	11	13
90	5.4913	5.4979	5.5045	5.5111	5.5177	5.5243	5.5309	5.5375	5.5441	5.5507	3	5	6	11	13
91	5.4979	5.5045	5.5111	5.5177	5.5243	5.5309	5.5375	5.5441	5.5507	5.5573	3	5	6	11	13
92	5.5045	5.5111	5.5177	5.5243	5.5309	5.5375	5.5441	5.5507	5.5573	5.5639	3	5	6	11	13
93	5.5111	5.5177	5.5243	5.5309	5.5375	5.5441	5.5507	5.5573	5.5639	5.5705	3	5	6	11	13
94	5.5177	5.5243	5.5309	5.5375	5.5441	5.5507	5.5573	5.5639	5.5705	5.5771	3	5	6	11	13
95	5.5243	5.5309	5.5375	5.5441	5.5507	5.5573	5.5639	5.5705	5.5771	5.5837	3	5	6	11	13
96	5.5309	5.5375	5.5441	5.5507	5.5573	5.5639	5.5705	5.5771	5.5837	5.5903	3	5	6	11	13
97	5.5375	5.5441	5.5507	5.5573	5.5639	5.5705	5.5771	5.5837	5.5903	5.5969	3	5	6	11	13
98	5.5441	5.5507	5.5573	5.5639	5.5705	5.5771	5.5837	5.5903	5.5969	5.6035	3	5	6	11	13
99	5.5507	5.5573	5.5639	5.5705	5.5771	5.5837	5.5903	5.5969	5.6035	5.6101	3	5	6	11	13
99.5	5.5573	5.5639	5.5705	5.5771	5.5837	5.5903	5.5969	5.6035	5.6101	5.6167	3	5	6	11	13
99.6	5.5639	5.5705	5.5771	5.5837	5.5903	5.5969	5.6035	5.6101	5.6167	5.6233	3	5	6	11	13
99.7	5.5705	5.5771	5.5837	5.5903	5.5969	5.6035	5.6101	5.6167	5.6233	5.6299	3	5	6	11	13
99.8	5.5771	5.5837	5.5903	5.5969	5.6035	5.6101	5.6167	5.6233	5.6299	5.6365	3	5	6	11	13
99.9	5.5837	5.5903	5.5969	5.6035	5.6101	5.6167	5.6233	5.6299	5.6365	5.6431	3	5	6	11	13
99.95	5.5903	5.5969	5.6035	5.6101	5.6167	5.6233	5.6299	5.6365	5.6431	5.6497	3	5	6	11	13

GH signal study IGF-1 data

Data for subjects JLMP

(normal IGF-1 value for stage 5 puberty: median 1.11 U/ml (0.53-2.04))

Study Time clock time mins	Pulse mean IGF-1 (SD) U/ml	Continuous mean IGF-1 (SD) U/ml	Control reference mean IGF-1 (SD)	p value (by ANOVA)
20.00h 0	1.21 (0.39)	1.37 (0.59)	0.95 (0.22)	
22.00h 120	1.07 (0.04)	1.31 (0.20)	0.99 (0.26)	
24.00h 240	1.06 (0.08)	0.96 (0.17)	0.94 (0.12)	
02.00h 360	1.12 (0.22)*	0.85 (0.24)	0.95 (0.20)	p=0.025*
04.00h 480	1.11 (0.16)*	0.95 (0.23)	0.92 (0.25)	p=0.006
06.00h 600	0.98 (0.26)	1.02 (0.42)	0.99 (0.14)	
08.00h 720	0.92 (0.37)	0.94 (0.41)	0.96 (0.10)	

Subject mean overnight 20-08.00h	Pulse mean IGF-1 (SD) U/ml	Continuous mean IGF-1 (SD) U/ml	Control reference mean IGF-1 (SD)	p value (paired t test)
J	1.08 (0.12)	1.00 (0.11)	1.10 (0.05)	
L	0.94 (0.13)	0.78 (0.20)	0.97 (0.21)	
M	0.92 (0.21)	1.07 (0.56)	0.89 (0.08)	
P	1.34 (0.22)	1.37 (0.17)	0.88 (0.20)	
mean	1.07 (0.09)*	1.06 (0.20)	0.96 (0.02)	p=0.02*

GH signal study
Cortisol data

Data for subject J

Study Time clock time mins	Pulse	Continuous	Control reference	p value (by ANOVA)
21.00h -60	218	489	219	
20.00h 0	145	246	138	
21.00h 60	231	131	154	
22.00h 120	143	82	76	
23.00h 180	102	56	61	
24.00h 240	60	40	50	
01.00h 300	67	<27	130	
02.00h 360	44	83	296	
03.00h 420	42	44	131	
04.00h 480	155	32	87	
05.00h 540	307	183	78	
06.00h 600	261	400	376	
07.00h 660	434	373	326	
08.00h 720	549	462	542	

Subject mean overnight 20-08.00h	Pulse	Continuous	Control reference	p value (paired t test)
J				
L				
M				
P				
mean				

LIST OF REFERENCES

- Achermann JC, Hindmarsh PC, Robinson IC, Matthews DR, Brook CG. (1999) The relative roles of continuous growth hormone-releasing hormone (GHRH(1-29)NH₂) and intermittent somatostatin(1-14)(SS) in growth hormone (GH) pulse generation: studies in normal and post cranial irradiated individuals. *Clin Endocrinol (Oxf)*. 1999 Nov;51(5):575-85.
- Adamson U, Efendic S, Lins PE, Wajngot A. (1982) Effect of somatostatin on insulin-induced hypoglycemia in man. *Acta Med Scand*. 1982;212(1-2):33-7.
- Agrawal AK, Shapiro BH. (2001) Intrinsic signals in the sexually dimorphic circulating growth hormone profiles of the rat. *Mol Cell Endocrinol*. 2001 Feb 28;173(1-2):167-81.
- Ajlouni K, Martinson DR, Hagen TC. (1975) Effect of glucose on the growth hormone response to L-dopa in normal and diabetic subjects. *Diabetes*. 1975 Jul;24(7):633-6.
- Alberti KG, Dornhorst A, Rowe AS. (1975) Metabolic rhythms in normal and diabetic man. Studies in insulin-treated diabetes. *Isr J Med Sci*. 1975 Jun;11(6):571-80.
- Albertsson-Wikland K, Rosberg S, Libre E, Lundberg LO, Groth T. (1989) Growth hormone secretory rates in children as estimated by deconvolution analysis of 24-h plasma concentration profiles. *Am J Physiol*. 1989 Dec;257(6 Pt 1):E809-14.
- Altman DG. (1991) Categorising continuous variables. *Br J Cancer*. 1991 Nov;64(5):975.
- Amiel SA, Gertner JM, Hintz RL, Press CM, Sherwin RS, Tamborlane WV. (1984) Effect of diabetes and its control on insulin-like growth factors in the young subject with type I diabetes. *Diabetes*. 1984 Dec;33(12):1175-9.
- Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV. (1986) Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med*. 1986 Jul 24;315(4):215-9.
- Amiel SA. (1991) "Brittle" diabetes. *BMJ*. 1991 Aug 3;303(6797):260-1.
- Amit T, Youdim MB, Hochberg Z. (2000) Clinical review 112: Does serum growth hormone (GH) binding protein reflect human GH receptor function? *J Clin Endocrinol Metab*. 2000 Mar;85(3):927-32. Review.

Arias P, Kerner W, de la Fuente A, Pfeiffer EF. (1984) Abnormal growth hormone levels in insulin-dependent diabetic patients under continuous subcutaneous insulin infusion and intensified conventional treatment. *Acta Endocrinol (Copenh)*. 1984 Oct;107(2):250-5.

Arslanian S, Ohki Y, Becker DJ, Drash AL. (1992) The dawn phenomenon: comparison between normal and insulin-dependent diabetic adolescents. *Pediatr Res*. 1992 Mar;31(3):203-6.

Asada N, Takahashi Y, Honjo M. (2000) Effects of 22K or 20K human growth hormone on lipolysis, leptin production in adipocytes in the presence and absence of human growth hormone binding protein. *Horm Res*. 2000;54(4):203-7.

Asplin CM, Faria AC, Carlsen EC, Vaccaro VA, Barr RE, Iranmanesh A, Lee MM, Veldhuis JD, Evans WS. (1989) Alterations in the pulsatile mode of growth hormone release in men and women with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*. 1989 Aug;69(2):239-45.

Astorga R, Cordido F, Casanueva FF, Dieguez C, Garcia-Mayor RV, Pombo M, Pombo CM, Popovic V. (1999) Regulation of growth hormone secretion by signals produced by the adipose tissue. *J Endocrinol Invest*. 1999;22(5 Suppl):22-6.

Atica JA, Luzio S, Owens DR. (1992) The dawn phenomenon and diabetes control in treated NIDDM and IDDM patients. *Diabetes Res Clin Pract*. 1992 Jun;16(3):183-90.

Avasthy N, Khokher MA, Taylor AM, Dandona P. (1986) Human plasma fractions inhibit the action of insulin on adipocytes and somatomedin on cartilage. *Diabetologia*. 1986 Jul;29(7):453-6.

Avogaro A, Burlina A, Calo L, Del Prato S, DeKreutzenberg S, Miola M, Maran A, Mingardi R, Piarulli F, Tiengo A. (1999) Effect of acute ketosis on the endothelial function of type 1 diabetic patients: the role of nitric oxide. *Diabetes*. 1999 Feb;48(2):391-7.

Balasse EO, Fery F. (1998) Ketone body production and disposal: effects of fasting, diabetes, and exercise. *Diabetes Metab Rev*. 1989 May;5(3):247-70. Review.

Balkau B, Cahane M, Caces E, Eschwege E, Tichet J, Vol S. (1998) Insulin dose and cardiovascular risk factors in type 1 diabetic children and adolescents. *Diabetes Metab*. 1998 Apr;24(2):143-50.

Barinaga M, Bilezikjian LM, Vale WW, Rosenfeld MG, Evans RM. (1985) Independent effects of growth hormone releasing factor on growth hormone release and gene transcription. *Nature*. 1985 Mar 21-27;314(6008):279-81.

Batch JA, Werther GA. (1992) Changes in growth hormone concentrations during puberty in adolescents with insulin dependent diabetes. *Clin Endocrinol (Oxf)*. 1992 Apr;36(4):411-6

Batchelor BR, Penner J, Hirsch J, Stern JS. (1976) Effects of hypophysectomy and acute growth hormone treatment upon glucose metabolism in adipose tissues and isolated adipocytes of rats. *Horm Metab Res*. 1976 Jan;8(1):24-33.

Baud L, Fouqueray B, Bellocq A, Doublier S, Dumoulin A. (1999) Growth hormone and somatostatin in glomerular injury. *J Nephrol*. 1999 Jan-Feb;12(1):18-23. Review.

Baumann G. (1979) Metabolic clearance rates of isohormones of human growth hormone in man. *J Clin Endocrinol Metab*. 1979 Oct;49(4):495-9.

Baumann G, Stolar MW, Buchanan TA. (1985) Slow metabolic clearance rate of the 20,000-dalton variant of human growth hormone: implications for biological activity. *Endocrinology*. 1985 Oct;117(4):1309-13.

Baumann G, Stolar MW, Buchanan TA. (1986) The metabolic clearance, distribution, and degradation of dimeric and monomeric growth hormone (GH): implications for the pattern of circulating GH forms. *Endocrinology*. 1986 Oct;119(4):1497-501.

Baumann G, Amburn KD, Buchanan TA. (1987) The effect of circulating growth hormone-binding protein on metabolic clearance, distribution, and degradation of human growth hormone. *J Clin Endocrinol Metab*. 1987 Apr;64(4):657-60.

Baumann G, Shaw MA. (1988) Immunochemical similarity of the human plasma growth hormone-binding protein and the rabbit liver growth hormone receptor. *Biochem Biophys Res Commun*. 1988 Apr 29;152(2):573-8.

Baumann G. (1990) Growth hormone binding proteins and various forms of growth hormone: implications for measurements. *Acta Paediatr Scand Suppl*. 1990;370:72-80; discussion 81. Review.

Baumann G. (1994) Growth hormone-binding proteins: state of the art. *J Endocrinol*. 1994 Apr;141(1):1-6. Review.

Baumann G. (2001) Growth hormone binding protein 2001. *J Pediatr Endocrinol Metab*. 2001 Apr;14(4):355-75. Review.

Baumbach WR, Horner DL, Logan JS. (1989) The growth hormone-binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor. *Genes Dev.* 1989 Aug;3(8):1199-205.

Baxter RC, Bryson JM, Turtle JR. (1980a) Somatogenic receptors of rat liver: regulation by insulin. *Endocrinology.* 1980 Oct;107(4):1176-81.

Baxter RC, Brown AS, Turtle JR. (1980b) Association between serum insulin, serum somatomedin and liver receptors for human growth hormone in streptozotocin diabetes. *Horm Metab Res.* 1980 Aug;12(8):377-81.

Baxter RC, Zaltsman Z. (1984) Induction of hepatic receptors for growth hormone (GH) and prolactin by GH infusion is sex independent. *Endocrinology.* 1984 Nov;115(5):2009-14.

Baxter RC, Martin JL, Tyler MI, Howden ME. (1986) Growth hormone-dependent insulin-like growth factor (IGF) binding protein from human plasma differs from other human IGF binding proteins. *Biochem Biophys Res Commun.* 1986 Sep 30;139(3):1256-61

Baxter RC, Martin JL. (1987) Binding proteins for insulin-like growth factors in adult rat serum. Comparison with other human and rat binding proteins. *Biochem Biophys Res Commun.* 1987 Aug 31;147(1):408-15.

Beaufrere B, Beylot M, Metz C, Ruitton A, Francois R, Riou JP, Mornex R. (1988) Dawn phenomenon in type 1 (insulin-dependent) diabetic adolescents: influence of nocturnal growth hormone secretion. *Diabetologia.* 1988 Aug;31(8):607-11.

Bercu BB, Diamond FB Jr. (1986) Growth hormone neurosecretory dysfunction. *Clin Endocrinol Metab.* 1986 Aug;15(3):537-90. Review.

Bereket A, Lang CH, Wilson TA. (1999) Alterations in the growth hormone-insulin-like growth factor axis in insulin dependent diabetes mellitus. *Horm Metab Res.* 1999 Feb-Mar;31(2-3):172-81.

Bercelowitz M, Szabo M, Frohman LA, Firestone S, Chu L, Hintz RL. (1981) Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science.* 1981 Jun 12;212(4500):1279-81

Bercelowitz M, Xu Y, Song J, Bruno JF. (1995) Regulation of somatostatin receptor mRNA expression. *Ciba Found Symp.* 1995;190:111-22; discussion 122-6.

Bergendahl M, Evans WS, Pastor C, Patel A, Iranmanesh A, Veldhuis JD. (1999) Short-term fasting suppresses leptin and (conversely) activates disorderly growth hormone secretion in midluteal phase women--a clinical research center study. *J Clin Endocrinol Metab.* 1999 Mar;84(3):883-94.

Berglund L, Berne C, Wollesen F (1999) Plasma endothelin-1 and total insulin exposure in diabetes mellitus. *Clin Sci (Lond)*. 1999 Aug;97(2):149-56.

Bergman RN, Ader M. (2000a) Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab*. 2000 Nov;11(9):351-6. Review.

Bergman RN. (2000b) Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein *Diabetologia*. 2000 Jul;43(7):946-52. Review.

Berneis K, Keller U. (1996) Metabolic actions of growth hormone: direct and indirect. *Baillieres Clin Endocrinol Metab*. 1996 Jul;10(3):337-52. Review.

Bick T, Amit T, Barkey RJ, Hertz P, Youdim MB, Hochberg Z. (1990) The interrelationship of growth hormone (GH), liver membrane GH receptor, serum GH-binding protein activity, and insulin-like growth factor I in the male rat. *Endocrinology*. 1990 Apr;126(4):1914-20.

Bick T, Hochberg Z, Amit T, Isaksson OG, Jansson JO. (1992) Roles of pulsatility and continuity of growth hormone (GH) administration in the regulation of hepatic GH-receptors, and circulating GH-binding protein and insulin-like growth factor-I. *Endocrinology*. 1992 Jul;131(1):423-9.

Bisschop PH, de Metz J, Ackermans MT, Endert E, Pijl H, Kuipers F, Meijer AJ, Sauerwein HP, Romijn JA. (2001) Dietary fat content alters insulin-mediated glucose metabolism in healthy men. *Am J Clin Nutr*. 2001 Mar;73(3):554-9.

Bizot-Espiard JG, Double A, Guardiola-Lemaitre B, Delagrangé P, Ktorza A, Penicaud L. (1998) Diurnal rhythms in plasma glucose, insulin, growth hormone and melatonin levels in fasted and hyperglycaemic rats. *Diabetes Metab*. 1998 Jun;24(3):235-40.

Bluet-Pajot MT, Epelbaum J, Gourdji D, Hammond C, Kordon C. (1998) Hypothalamic and hypophyseal regulation of growth hormone secretion. *Cell Mol Neurobiol*. 1998 Feb;18(1):101-23. Review

Boden G.(2001) Free fatty acids-the link between obesity and insulin resistance. *Endocr Pract*. 2001 Jan;7(1):44-51.

Boden G. (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes*. 1997 Jan;46(1):3-10. Review.

Boer P. (1984) Estimated lean body mass as an index for normalization of body fluid volumes in humans. *Am J Physiol*. 1984 Oct;247(4 Pt 2):F632-6.

Boisclair YR, Johnston KB, Bauman DE, Crooker BA, Dunshea FR, Bell AW. (1997) Paradoxical increases of circulating non-esterified fatty acids in

somatotropin treated cattle undergoing mild disturbances. *Domest Anim Endocrinol.* 1997 Jul;14(4):251-62.

Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Calcinaro F, Lolli C, Campbell P, Brunetti P, Gerich JE. (1984a) Demonstration of a dawn phenomenon in normal human volunteers. *Diabetes.* 1984 Dec;33(12):1150-3.

Bolli GB, Gerich JE. (1984b) The "dawn phenomenon"--a common occurrence in both non-insulin-dependent and insulin-dependent diabetes mellitus. *N Engl J Med.* 1984 Mar 22;310(12):746-50.

Bolli GB. (1988) The dawn phenomenon: its origin and contribution to early morning hyperglycemia in diabetes mellitus. *Diabete Metab.* 1988 Dec;14(6):675-86. Review.

Bonadonna RC, Zych K, Boni C, Ferrannini E, DeFronzo RA. (1989) Time dependence of the interaction between lipid and glucose in humans. *Am J Physiol.* 1989 Jul;257(1 Pt 1):E49-56.

Boucher BJ, Butterfield WJ, Whichelow MJ. (1969) The disappearance of labelled human growth hormone from the forearm circulation in normal and diabetic subjects. *Clin Sci.* 1969 Dec;37(3):721-30.

Bougneres PF, Artavia-Loria E, Ferre P, Chaussain JL, Job JC. (1985) Effects of hypopituitarism and growth hormone replacement therapy on the production and utilization of glucose in childhood. *J Clin Endocrinol Metab.* 1985 Dec;61(6):1152-7.

Bowden CR, Bergman RN, Marsh DJ. (1980) Cause of glucose oscillations during glucose infusion: periodic variation in glucose uptake. *Am J Physiol.* 1980 Apr;238(4):E395-407.

Bowsher RR, Apathy JM, Ferguson AL, Riggan RM, Henry DP. (1990) Cross-reactivity of monomeric and dimeric biosynthetic human growth hormone in commercial immunoassays. *Clin Chem.* 1990 Feb;36(2):362-6.

Boyle PJ, Avogaro A, Smith L, Shah SD, Cryer PE, Santiago JV. (1992) Absence of the dawn phenomenon and abnormal lipolysis in type 1 (insulin-dependent) diabetic patients with chronic growth hormone deficiency. *Diabetologia.* 1992 Apr;35(4):372-9.

Brain CE, Brook CG, Hindmarsh PC, Matthews DR, Robinson IC. (1991) The interaction of growth hormone releasing hormone and somatostatin in the generation of a GH pulse in man. *Clin Endocrinol (Oxf).* 1991 Oct;35(4):353-60.

Brandenberger G, Gronfier C, Chapotot F, Simon C, Piquard F. (2000) Effect of sleep deprivation on overall 24 h growth-hormone secretion. *Lancet*. 2000 Oct 21;356(9239):1408.

Bratusch-Marrain PR, Komjati M, Waldhausl WK. (1986a) Efficacy of pulsatile versus continuous insulin administration on hepatic glucose production and glucose utilization in type I diabetic humans. *Diabetes*. 1986 Aug;35(8):922-6.

Bratusch-Marrain PR, DeFronzo RA. (1986b) Failure of hyperketonemia to alter basal and insulin-mediated glucose metabolism in man. *Horm Metab Res*. 1986 Mar;18(3):185-9.

Brazeau P, Gullemain R. (1974) Editorial: Somatostatin: newcomer from the hypothalamus. *N Engl J Med*. 1974 Apr 25;290(17):963-4.

Breier BH, Bass JJ, Butler JH, Gluckman PD. (1986) The somatotrophic axis in young steers: influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor 1. *J Endocrinol*. 1986 Nov;111(2):209-15.

Brennan JR, Gebhart SS, Blackard WG. (1985) Pump-induced insulin aggregation. A problem with the Biostator. *Diabetes*. 1985 Apr;34(4):353-9.

Bright GM, Veldhuis JD, Iranmanesh A, Baumann G, Maheshwari H, Lima J (1999) Appraisal of growth hormone (GH) secretion: evaluation of a composite pharmacokinetic model that discriminates multiple components of GH input. *J Clin Endocrinol Metab*. 1999 Sep;84(9):3301-8. PMID: 10487702; UI: 99415643

Bristow AF, Jeffcoate SL. (1992) Analysis of therapeutic growth hormone preparations: report of an interlaboratory collaborative study on growth hormone assay methodologies. *Biologicals*. 1992 Sep;20(3):221-31.

Bruno JF, Xu Y, Song J, Berelowitz M. (1994) Pituitary and hypothalamic somatostatin receptor subtype messenger ribonucleic acid expression in the food-deprived and diabetic rat. *Endocrinology*. 1994 Nov;135(5):1787-92.

Burge MR, Hardy KJ, Schade DS. (1993) Short-term fasting is a mechanism for the development of euglycemic ketoacidosis during periods of insulin deficiency. *J Clin Endocrinol Metab*. 1993 May;76(5):1192-8.

Busiguina S, Argente J, Garcia-Segura LM, Chowen JA. (2000) Anatomically specific changes in the expression of somatostatin, growth hormone-releasing hormone and growth hormone receptor mRNA in diabetic rats. *J Neuroendocrinol*. 2000 Jan;12(1):29-39.

Calabresi E, Ishikawa E, Bartolini L, Delitala G, Fanciulli G, Oliva O, Veldhuis JD, Serio M. (1996) Somatostatin infusion suppresses GH secretory

burst frequency and mass in normal men. *Am J Physiol.* 1996 Jun;270(6 Pt 1):E975-9.

Campbell PJ, Bolli GB, Cryer PE, Gerich JE. (1985a) Sequence of events during development of the dawn phenomenon in insulin-dependent diabetes mellitus. *Metabolism.* 1985 Dec;34(12):1100-4.

Campbell PJ, Bolli GB, Cryer PE, Gerich JE. (1985b) Pathogenesis of the dawn phenomenon in patients with insulin-dependent diabetes mellitus. Accelerated glucose production and impaired glucose utilization due to nocturnal surges in growth hormone secretion. *N Engl J Med.* 1985 Jun 6;312(23):1473-9.

Campbell PJ, Gerich JE. (1986) Occurrence of dawn phenomenon without change in insulin clearance in patients with insulin-dependent diabetes mellitus. *Diabetes.* 1986 Jul;35(7):749-52.

Caprio S, Plewe G, Diamond MP, Simonson DC, Boulware SD, Sherwin RS, Tamborlane WV. (1989) Increased insulin secretion in puberty: a compensatory response to reductions in insulin sensitivity. *J Pediatr.* 1989 Jun;114(6):963-7.

Caregaro L, Favaro A, Santonastaso P, Alberino F, Di Pascoli L, Nardi M, Favaro S, Gatta A. (2001) Insulin-like growth factor 1 (IGF-1), a nutritional marker in patients with eating disorders. *Clin Nutr.* 2001 Jun;20(3):251-7.

Carpentier V, Vaudry H, Laquerriere A, Tayot J, Leroux P. (1996) Distribution of somatostatin receptors in the adult human brainstem. *Brain Res.* 1996 Sep 23;734(1-2):135-48.

Carrel AL, Allen DB. (2000) Effects of growth hormone on adipose tissue. *J Pediatr Endocrinol Metab.* 2000 Sep;13 Suppl 2:1003-9

Carter-Su C, King AP, Argetsinger LS, Smit LS, Vanderkuur J, Campbell GS. (1996) Signalling pathway of GH. *Endocr J.* 1996 Oct;43 Suppl:S65-70. Review.

Casanueva FF, Villanueva L, Dieguez C, Diaz Y, Cabranes JA, Szoke B, Scanlon MF, Schally AV, Fernandez-Cruz A. (1987) Free fatty acids block growth hormone (GH) releasing hormone-stimulated GH secretion in man directly at the pituitary. *J Clin Endocrinol Metab.* 1987 Oct;65(4):634-42.

Castillo MJ, Scheen AJ, Letiexhe MR, Lefebvre PJ. (1994) How to measure insulin clearance. *Diabetes Metab Rev.* 1994 Jul;10(2):119-50. Review.

Ceda GP, Hoffman AR, Silverberg GD, Wilson DM, Rosenfeld RG. (1985) Regulation of growth hormone release from cultured human pituitary

adenomas by somatomedins and insulin. *J Clin Endocrinol Metab.* 1985 Jun;60(6):1204-9.

Ceda GP, Davis RG, Rosenfeld RG, Hoffman AR. (1987) The growth hormone (GH)-releasing hormone (GHRH)-GH-somatomedin axis: evidence for rapid inhibition of GHRH-elicited GH release by insulin-like growth factors I and II. *Endocrinology.* 1987 Apr;120(4):1658-62.

Celniker AC, Chen AB, Wert RM Jr, Sherman BM. (1989) Variability in the quantitation of circulating growth hormone using commercial immunoassays. *J Clin Endocrinol Metab.* 1989 Feb;68(2):469-76.

Chambrier C, Picard S, Vidal H, Cohen R, Riou JP, Beylot M. (1990) Interactions of glucagon and free fatty acids with insulin in control of glucose metabolism. *Metabolism.* 1990 Sep;39(9):976-84.

Chapman IM, Helfgott A, Willoughby JO. (1991) Disappearance half-life times of exogenous and growth hormone-releasing factor-stimulated endogenous growth hormone in normal rats. *J Endocrinol.* 1991 Mar;128(3):369-74.

Chatfield C (1975) The analysis of time series: Theory and Practice. Chapman and Hall, London.

Chen NY, Chen WY, Kopchick JJ. (1997) Liver and kidney growth hormone (GH) receptors are regulated differently in diabetic GH and GH antagonist transgenic mice. *Endocrinology.* 1997 May;138(5):1988-94.

Cherrington AD, Edgerton D, Sindelar DK. (1998) The direct and indirect effects of insulin on hepatic glucose production in vivo. *Diabetologia.* 1998 Sep;41(9):987-96. Review.

Clarke WL, Haymond MW, Santiago JV. (1980) Overnight basal insulin requirements in fasting insulin-dependent diabetics. *Diabetes.* 1980 Jan;29(1):78-80.

Clasey JL, Weltman A, Patrie J, Weltman JY, Pezzoli S, Bouchard C, Thorner MO, Hartman ML. (2001) Abdominal visceral fat and fasting insulin are important predictors of 24-hour GH release independent of age, gender, and other physiological factors. *J Clin Endocrinol Metab.* 2001 Aug;86(8):3845-52.

Clayton KL, Cheetham TD, Carlsson LM, Dunger DB, Holly JM, Jones J, Taylor AM. (1994) Loss of the normal relationships between growth hormone, growth hormone-binding protein and insulin-like growth factor-I in adolescents with insulin-dependent diabetes mellitus. *Clin Endocrinol (Oxf).* 1994 Oct;41(4):517-24.

Clayton RN, Royston JP, Chapman J, Wilson M, Obhrai M, Sawers RS, Lynch SS. (1987) Is changing hypothalamic activity important for control of ovulation? *Br Med J (Clin Res Ed).* 1987 Jul 4;295(6589):7-12.

- Clemens AH, Hough DL, D'Orazio PA. (1982) Development of the Biostator Glucose clamping algorithm. *Clin Chem.* 1982 Sep;28(9):1899-904.
- Cohen RM, Abplanalp WA. (1991) Resistance of growth hormone secretion to somatostatin in men with type I diabetes mellitus. *Diabetes.* 1991 Oct;40(10):1251-8.
- Coiro V, Passeri M, Gardini E, Capretti L, Speroni G, Davoli C, Maffei ML, Fagnoni F, Bianconi L, Volpi R, et al. (1990) Reduced sensitivity to pirenzepine-induced blockade of growth hormone responses to arginine, exercise, and growth hormone-releasing hormone in type I diabetic subjects. *Metabolism.* 1990 Jul;39(7):668-75.
- Collins AC, Pickup JC. (1985) Sample preparation and radioimmunoassay for circulating free and antibody-bound insulin concentrations in insulin-treated diabetics: a re-evaluation of methods. *Diabet Med.* 1985 Nov;2(6):456-60.
- Cronin MJ, Thorner MO, Weiss J. (1987) Periodic interactions of GH-releasing factor and somatostatin can augment GH release in vitro. *Am J Physiol.* 1987 Nov;253(5 Pt 1):E508-14.
- Csaba Z, Dournaud P. (2001) Cellular biology of somatostatin receptors. *Neuropeptides.* 2001 Feb;35(1):1-23. Review.
- Cummings EA, Dekker MG, Daneman D, Lawson ML, Sochett EB. (1998) Contribution of growth hormone and IGF-I to early diabetic nephropathy in type 1 diabetes. *Diabetes.* 1998 Aug;47(8):1341-6.
- Cuneo RC, Hickman PE, Wallace JD, Teh BT, Ward G, Veldhuis JD, Waters MJ. (1995) Altered endogenous growth hormone secretory kinetics and diurnal GH-binding protein profiles in adults with chronic liver disease. *Clin Endocrinol (Oxf).* 1995 Sep;43(3):265-75.
- Cunha SR, DeAlmeida V, Godfrey P, Mayo KE, Miller T, Zheng J. (2000) Regulation of the pituitary somatotroph cell by GHRH and its receptor. *Recent Prog Horm Res.* 2000;55:237-66; discussion 266-7. Review.
- Davidson MB, Harris MD, Ziel FH, Rosenberg CS. (1988) Suppression of sleep-induced growth hormone secretion by anticholinergic agent abolishes dawn phenomenon. *Diabetes.* 1988 Feb;37(2):166-71.
- Davidson MB. (1987) Effect of growth hormone on carbohydrate and lipid metabolism. *Endocr Rev.* 1987 May;8(2):115-31. Review.

Davis MR, Shamoon H. (1992) Impaired glucose disposal following mild hypoglycemia in nondiabetic and type I diabetic humans. *Metabolism*. 1992 Feb;41(2):216-23.

Dawson JM, Greathead HM, Craigon J, Hachey DL, Reeds PJ, Pell JM, Buttery PJ. (1998) The interaction between nutritional status and growth hormone in young cattle: differential responsiveness of fat and protein metabolism. *Br J Nutr*. 1998 Mar;79(3):275-86.

De Feo P, Perriello G, Ventura MM, Calcinaro F, Basta G, Lolli C, Cruciani C, Dell'Olio A, Santeusano F, Brunetti P, et al. (1986) Studies on overnight insulin requirements and metabolic clearance rate of insulin in normal and diabetic man: relevance to the pathogenesis of the dawn phenomenon. *Diabetologia*. 1986 Aug;29(8):475-80.

DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J. (1983) Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes*. 1983 Jan;32(1):35-45.

DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. (1985) Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest*. 1985 Jul;76(1):149-55.

DeFronzo RA, Hendler R, Christensen N. (1980) Stimulation of counterregulatory hormonal responses in diabetic man by a fall in glucose concentration. *Diabetes*. 1980 Feb;29(2):125-31.

DeFronzo RA, Tobin JD, Andres R. (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979 Sep;237(3):E214-23.

Dejgaard A, Hilsted J, Henriksen JH, Christensen NJ. (1989) Plasma adrenaline kinetics in type 1 (insulin-dependent) diabetic patients with and without autonomic neuropathy. *Diabetologia*. 1989 Nov;32(11):810-3.

Del Prato S, Matsuda M, Simonson DC, Groop LC, Sheehan P, Leonetti F, Bonadonna RC, DeFronzo RA. (1997) Studies on the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance. *Diabetologia*. 1997 Jun;40(6):687-97.

Dieguez C, Page MD, Scanlon MF. (1988) Growth hormone neuroregulation and its alterations in disease states. *Clin Endocrinol (Oxf)*. 1988 Jan;28(1):109-43. Review

Dietz J, Schwartz J. (1991) Growth hormone alters lipolysis and hormone-sensitive lipase activity in 3T3-F442A adipocytes. *Metabolism*. 1991 Aug;40(8):800-6.

Dimaraki EV, Jaffe CA, Demott-Friberg R, Russell-Aulet M, Bowers CY, Marbach P, Barkan AL. (2001) Generation of growth hormone pulsatility in women: evidence against somatostatin withdrawal as pulse initiator. *Am J Physiol Endocrinol Metab*. 2001 Mar;280(3):E489-95.

DiStefano JJ 3rd, Malone TK, Jang M. (1982) Comprehensive kinetics of thyroxine distribution and metabolism in blood and tissue pools of the rat from only six blood samples: dominance of large, slowly exchanging tissue pools. *Endocrinology*. 1982 Jul;111(1):108-17.

Donaldson DL, Pan F, Hollowell JG, Stevenson JL, Gifford RA, Moore WV. (1981) Reliability of stimulated and spontaneous growth hormone (GH) levels for identifying the child with low GH secretion. *J Clin Endocrinol Metab*. 1991 Mar;72(3):647-52.

Dournaud P, Csaba Z. (2001) Cellular biology of somatostatin receptors. *Neuropeptides*. 2001 Feb;35(1):1-23.

Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI. (1999) Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest*. 1999 Jan;103(2):253-9.

Driver PM, Forbes JM. (1981) Episodic growth hormone secretion in sheep in relation to time of feeding, spontaneous meals and short term fasting. *J Physiol*. 1981 Aug;317:413-24.

Dunger DB, Edge JA, Pal R, Taylor AM, Holly JM, Matthews DR. (1991) Impact of increased growth hormone secretion on carbohydrate metabolism in adolescents with diabetes. *Acta Paediatr Scand Suppl*. 1991;377:69-77; discussion 78.

Dux S, White NH, Skor DA, Santiago JV. (1985) Insulin clearance contributes to the variability of nocturnal insulin requirement in insulin-dependent diabetes mellitus. *Diabetes*. 1985 Dec;34(12):1260-5.

Ebeling P, Koivisto VA, Stenman UH, Seppala M. (1995) Androgens and insulin resistance in type 1 diabetic men. *Clin Endocrinol (Oxf)*. 1995 Nov;43(5):601-7.

Edge JA, Dunger DB, Matthews DR, Gilbert JP, Smith CP. (1990a) Increased overnight growth hormone concentrations in diabetic compared with normal adolescents. *J Clin Endocrinol Metab*. 1990 Nov;71(5):1356-62.

Edge JA, Matthews DR, Dunger DB. (1990b) The dawn phenomenon is related to overnight growth hormone release in adolescent diabetics. *Clin Endocrinol (Oxf)*. 1990 Dec;33(6):729-37.

Edge JA, Harris DA, Phillips PE, Pal BR, Matthews DR, Dunger DB. (1993) Evidence for a role for insulin and growth hormone in overnight regulation of 3-hydroxybutyrate in normal and diabetic adolescents. *Diabetes Care*. 1993 Jul;16(7):1011-8.

Enjalbert A. (1989) Multiple transduction mechanisms of dopamine, somatostatin and angiotensin II receptors in anterior pituitary cells. *Horm Res*. 1989;31(1-2):6-12. Review.

Epelbaum J, Agid F, Agid Y, Beaudet A, Bertrand P, Enjalbert A, Heidet V, Kordon C, Krantic S, Leonard JF, et al. (1989) Somatostatin receptors in brain and pituitary. *Horm Res*. 1989;31(1-2):45-50. Review.

Eriksson J, Gause-Nilsson I, Lonnroth P, Smith U. (1990) The insulin-like effect of growth hormone on insulin-like growth factor II receptors is opposed by cyclic AMP. Evidence for a common post-receptor pathway for growth hormone and insulin action. *Biochem J*. 1990 Jun 1;268(2):353-7.

Evans WS, Vance ML, Kaiser DL, Sellers RP, Borges JL, Downs TR, Frohman LA, Rivier J, Vale W, Thorner MO. (1985b) Effects of intravenous, subcutaneous, and intranasal administration of growth hormone (GH)-releasing hormone-40 on serum GH concentrations in normal men. *J Clin Endocrinol Metab*. 1985 Nov;61(5):846-50.

Evans PJ, Dieguez C, Foord S, Peters JR, Hall R, Scanlon MF. (1985a) The effects of cholinergic blockade on the growth hormone and prolactin response to insulin hypoglycaemia. *Clin Endocrinol (Oxf)*. 1985 Jun;22(6):733-7.

Evans WS, Faria AC, Christiansen E, Ho KY, Weiss J, Rogol AD, Johnson ML, Blizzard RM, Veldhuis JD, Thorner MO. (1987) Impact of intensive venous sampling on characterization of pulsatile GH release. *Am J Physiol*. 1987 Apr;252(4 Pt 1):E549-56.

Fairhall KM, Gabrielsson BG, Robinson IC. (1990) Effect of food withdrawal and insulin on growth hormone secretion in the guinea pig. *Endocrinology*. 1990 Aug;127(2):716-23.

Faria AC, Veldhuis JD, Thorner MO, Vance ML. (1989) Half-time of endogenous growth hormone (GH) disappearance in normal man after stimulation of GH secretion by GH-releasing hormone and suppression with somatostatin. *J Clin Endocrinol Metab*. 1989 Mar;68(3):535-41.

Felig P, Wahren J, Sherwin R, Hendler R. (1976) Insulin, glucagon, and somatostatin in normal physiology and diabetes mellitus. *Diabetes*. 1976 Dec;25(12):1091-9.

Feneberg R, Sparber M, Veldhuis JD, Mehls O, Ritz E, Schaefer F. (1999) Synchronous fluctuations of blood insulin and lactate concentrations in humans. *J Clin Endocrinol Metab*. 1999 Jan;84(1):220-7.

Ferrannini E, Locatelli L, Jequier E, Felber JP. (1989) Differential effects of insulin and hyperglycemia on intracellular glucose disposition in humans. *Metabolism*. 1989 May;38(5):459-65.

Fery F, Balasse EO (1985) Ketone body production and disposal in diabetic ketosis. A comparison with fasting ketosis. *Diabetes* 1985 Apr;34(4):326-32

Fery F, Plat L, Melot C, Balasse EO. (1996) Role of fat-derived substrates in the regulation of gluconeogenesis during fasting. *Am J Physiol*. 1996 May;270(5 Pt 1):E822-30.

Finkelstein JW, Roffwarg HP, Boyar RM, Kream J, Hellman L. (1972) Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. *J Clin Endocrinol Metab*. 1972 Nov;35(5):665-70.

Florini JR, Ewton DZ, Coolican SA. (1996) Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev* 1996 Oct;17(5):481-517

Flyvbjerg A. (1997) Role of growth hormone, insulin-like growth factors (IGFs) and IGF-binding proteins in the renal complications of diabetes. *Kidney Int Suppl*. 1997 Sep;60:S12-9. Review.

Fowelin J, Attvall S, von Schenck H, Smith U, Lager I. (1991) Characterization of the insulin-antagonistic effect of growth hormone in man. *Diabetologia*. 1991 Jul;34(7):500-6.

Fowelin J, Attvall S, von Schenck H, Smith U, Lager I. (1995) Characterization of the insulin-antagonistic effect of growth hormone in insulin-dependent diabetes mellitus. *Diabet Med*. 1995 Nov;12(11):990-6.

Francis AJ, Home PD, Hanning I, Alberti KG, Tunbridge WM. (1983) Intermediate acting insulin given at bedtime: effect on blood glucose concentrations before and after breakfast. *Br Med J (Clin Res Ed)*. 1983 Apr 9;286(6372):1173-6.

Frohman LA. (1983) CNS peptides and glucoregulation. *Annu Rev Physiol*. 1983;45:95-107. Review.

Fryklund L. (1987) Production of authentic recombinant somatropin. *Acta Paediatr Scand Suppl*. 1987;331:5-8.

Fukata J, Diamond DJ, Martin JB. (1985) Effects of rat growth hormone (rGH)-releasing factor and somatostatin on the release and synthesis of rGH in dispersed pituitary cells. *Endocrinology*. 1985 Aug;117(2):457-67.

Garcia-Mayor RV, Perez AJ, Gandara A, Andrade A, Mallo F, Casanueva FF. (1993) Metabolic clearance rate of biosynthetic growth hormone after endogenous growth hormone suppression with a somatostatin analogue in chronic renal failure patients and control subjects. *Clin Endocrinol (Oxf)*. 1993 Sep;39(3):337-43.

Gardner MJ, Altman DG. (1986) Confidence intervals rather than P values: estimation rather than hypothesis testing. *Br Med J (Clin Res Ed)*. 1986 Mar 15;292(6522):746-50.

Gelato MC, Loriaux DL, Merriam GR, Oldfield E. (1990) Pulsatile growth hormone secretion in patients with acromegaly and normal men: the effects of growth hormone-releasing hormone infusion. *J Clin Endocrinol Metab*. 1990 Sep;71(3):585-90.

Gelato MC, Malozowski S, Caruso-Nicoletti M, Ross JL, Pescovitz OH, Rose S, Loriaux DL, Cassorla F, Merriam GR. (1986) Growth hormone (GH) responses to GH-releasing hormone during pubertal development in normal boys and girls: comparison to idiopathic short stature and GH deficiency. *J Clin Endocrinol Metab*. 1986 Jul;63(1):174-9.

Gerich JE, Lorenzi M, Karam JH, Schneider V, Forsham PH. (1975a) Abnormal pancreatic glucagon secretion and postprandial hyperglycemia in diabetes mellitus. *JAMA*. 1975 Oct 13;234(2):159-5.

Gerich JE, Lorenzi M, Hane S, Gustafson G, Guillemin R, Forsham PH. (1975b) Evidence for a physiologic role of pancreatic glucagon in human glucose homeostasis: studies with somatostatin. *Metabolism*. 1975 Feb;24(2):175-82.

Gerich JE, Lorenzi M, Bier DM, Tsalikian E, Schneider V, Karam JH, Forsham PH. (1976) Effects of physiologic levels of glucagon and growth hormone on human carbohydrate and lipid metabolism. Studies involving administration of exogenous hormone during suppression of endogenous hormone secretion with somatostatin. *J Clin Invest*. 1976 Apr;57(4):875-84.

Gerich J, Davis J, Lorenzi M, Rizza R, Bohannon N, Karam J, Lewis S, Kaplan R, Schultz T, Cryer P. (1979) Hormonal mechanisms of recovery from insulin-induced hypoglycemia in man. *Am J Physiol*. 1979 Apr;236(4):E380-5.

Gerich JE. (1986) Insulin-dependent diabetes mellitus: pathophysiology. *Mayo Clin Proc*. 1986 Oct;61(10):787-91

Getty L, Panteleon AE, Mittelman SD, Dea MK, Bergman RN. (2000) Rapid oscillations in omental lipolysis are independent of changing insulin levels in vivo. *J Clin Invest.* 2000 Aug;106(3):421-30.

Ghigo E, Goffi S, Mazza E, Arvat E, Procopio M, Bellone J, Muller EE, Camanni F. (1989) Repeated GH-releasing hormone administration unravels different GH secretory patterns in normal adults and children. *Acta Endocrinol (Copenh).* 1989 May;120(5):598-601

Giampietro O, Ferdeghini M, Miccoli R, Locatelli V, Cerri M, Yanaihara N, Navalesi R, Muller EE. (1987) Effect of growth hormone-releasing hormone and clonidine on growth hormone release in type 1 diabetic patients. *Horm Metab Res.* 1987 Dec;19(12):636-41.

Gianotti L, Fassino S, Daga GA, Lanfranco F, De Bacco C, Ramunni J, Arvat E, MacCario M, Ghigo E. (2000) Effects of free fatty acids and acipimox, a lipolysis inhibitor, on the somatotroph responsiveness to GHRH in anorexia nervosa. *Clin Endocrinol (Oxf).* 2000 Jun;52(6):713-20.

Giustina A, Bossoni S, Cimino A, Pizzocolo G, Romanelli G, Wehrenberg WB. (1990) Impaired growth hormone (GH) response to pyridostigmine in type 1 diabetic patients with exaggerated GH-releasing hormone-stimulated GH secretion. *J Clin Endocrinol Metab.* 1990 Dec;71(6):1486-90.

Giustina A, Bodini C, Bossoni S, Valentini U, Wehrenberg WB. (1993) Variability in the growth hormone response to growth hormone-releasing hormone alone or combined with pyridostigmine in type 1 diabetic patients. *J Endocrinol Invest.* 1993 Sep;16(8):585-90.

Gjedde A, Crone C. (1981) Blood-brain glucose transfer: repression in chronic hyperglycemia. *Science.* 1981 Oct 23;214(4519):456-7.

Goldbeter A, Dupont G, Halloy J. (2000) The frequency encoding of pulsatility. *Novartis Found Symp.* 2000;227:19-36; discussion 36-45. Review

Goni MJ, Monreal M, Goni F, Sopena M, Gil MJ, Moncada E, Salvador J. (1997) Effects of cholinergic blockade on nocturnal thyrotropin and growth hormone (GH) secretion in type I diabetes mellitus: further evidence supporting somatostatin's involvement in GH suppression. *Metabolism.* 1997 Nov;46(11):1305-11.

Goodman HM. (1970) Antilipolytic effects of growth hormone. *Metabolism.* 1970 Oct;19(10):849-55.

Goodman HM. (1981) Separation of early and late responses of adipose tissue to growth hormone. *Endocrinology.* 1981 Jul;109(1):120-9.

- Gorin E, Grichting G, Goodman HM. (1984) Binding and degradation of [125I]human growth hormone in rat adipocytes. *Endocrinology*. 1984 Aug;115(2):467-75.
- Gottesman I, Mandarino L, Gerich J. (1984) Use of glucose uptake and glucose clearance for the evaluation of insulin action in vivo. *Diabetes*. 1984 Feb;33(2):184-91.
- Grant PJ, Stickland MH, Bristow AF, Clarke RF, Wales JK. (1989) Insulin increases plasma somatomedin C (IGF-1) concentrations in adult type 1 diabetic patients. *Diabet Med*. 1989 Apr;6(3):245-8.
- Gravholt CH, Schmitz O, Simonsen L, Bulow J, Christiansen JS, Moller N. (1999) Effects of a physiological GH pulse on interstitial glycerol in abdominal and femoral adipose tissue. *Am J Physiol*. 1999 Nov;277(5 Pt 1):E848-54.
- Gravholt CH, Moller N, Jensen MD, Christiansen JS, Schmitz O. (2001) Physiological levels of glucagon do not influence lipolysis in abdominal adipose tissue as assessed by microdialysis. *J Clin Endocrinol Metab*. 2001 May;86(5):2085-9.
- Gray RS, Cowan P, Duncan LJ, Clarke BF. (1986) Reversal of insulin resistance in type 1 diabetes following initiation of insulin treatment. *Diabet Med*. 1986 Jan;3(1):18-23.
- Goran MI, Gower BA. (2001) Longitudinal study on pubertal insulin resistance. *Diabetes*. 2001 Nov;50(11):2444-50.
- Grunstein HS, James DE, Storlien LH, Smythe GA, Kraegen EW. (1985) Hyperinsulinemia suppresses glucose utilization in specific brain regions: in vivo studies using the euglycemic clamp in the rat. *Endocrinology*. 1985 Feb;116(2):604-10.
- Guler HP, Zapf J, Froesch ER. (1987) Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. *N Engl J Med*. 1987 Jul 16;317(3):137-40.
- Hachiya HL, Treves ST, Kahn CR, Sodoyez JC, Sodoyez-Goffaux F. (1987) Altered insulin distribution and metabolism in type I diabetics assessed by [123I]insulin scanning. *J Clin Endocrinol Metab*. 1987 Apr;64(4):801-8.
- Haffner D, Schaefer F, Girard J, Ritz E, Mehls O. (1994) Metabolic clearance of recombinant human growth hormone in health and chronic renal failure. *J Clin Invest*. 1994 Mar;93(3):1163-71.

Hagstrom-Toft E, Bolinder J, Ungerstedt U, Arner P. (1997) A circadian rhythm in lipid mobilization which is altered in IDDM. *Diabetologia*. 1997 Sep;40(9):1070-8.

Halldin MU, Tylleskar K, Hagenas L, Tuvemo T, Gustafsson J. (1998) Is growth hormone hypersecretion in diabetic adolescent girls also a daytime problem? *Clin Endocrinol (Oxf)*. 1998 Jun;48(6):785-94.

Hansen AP, Johansen K. (1970) Diurnal patterns of blood glucose, serum free fatty acids, insulin, glucagon and growth hormone in normals and juvenile diabetics. *Diabetologia*. 1970 Feb;6(1):27-33.

Harris MD, Davidson MB, Rosenberg CS. (1986) Simple solution to problem of biostator-induced insulin aggregation. *Diabetes Care*. 1986 Jul-Aug;9(4):356-8.

Hart IC, Chadwick PM, Boone TC, Langley KE, Rudman C, Souza LM. (1984) A comparison of the growth-promoting, lipolytic, diabetogenic and immunological properties of pituitary and recombinant-DNA-derived bovine growth hormone (somatotropin). *Biochem J*. 1984 Nov 15;224(1):93-100.

Hartman ML, Faria AC, Vance ML, Johnson ML, Thorner MO, Veldhuis JD. (1991) Temporal structure of in vivo growth hormone secretory events in humans. *Am J Physiol*. 1991 Jan;260(1 Pt 1):E101-10.

Hartman ML, Veldhuis JD, Johnson ML, Lee MM, Alberti KG, Samojlik E, Thorner MO. (1992) Augmented growth hormone (GH) secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. *J Clin Endocrinol Metab*. 1992 Apr;74(4):757-65.

Hartman ML, Pezzoli SS, Hellmann PJ, Suratt PM, Thorner MO. (1996) Pulsatile growth hormone secretion in older persons is enhanced by fasting without relationship to sleep stages. *J Clin Endocrinol Metab*. 1996 Jul;81(7):2694-701.

Hasselbalch SG, Knudsen GM, Jakobsen J, Hageman LP, Holm S, Paulson OB. (1995) Blood-brain barrier permeability of glucose and ketone bodies during short-term starvation in humans. *Am J Physiol*. 1995 Jun;268(6 Pt 1):E1161-6.

Hayford JT, Danney MM, Hendrix JA, Thompson RG. (1980) Integrated concentration of growth hormone in juvenile-onset diabetes. *Diabetes*. 1980 May;29(5):391-8.

Hellman B, Lernmark A. (1969) Inhibition of the in vitro secretion of insulin by an extract of pancreatic alpha-1 cells. *Endocrinology*. 1969 Jun;84(6):1484-8.

Hellman L, Nakada F, Curti J, Weitzman ED, Kream J, Roffwarg H, Ellman S, Fukushima DK, Gallagher TF. (1970) Cortisol is secreted episodically by normal man. *J Clin Endocrinol Metab.* 1970 Apr;30(4):411-22.

Hermansen K, Moller A, Christensen CK, Christiansen JS, Schmitz O, Orskov H, Alberti KG, Mogensen CE. (1987) Diurnal plasma profiles of metabolite and hormone concentration in insulin-dependent diabetic patients during conventional insulin treatment and continuous subcutaneous insulin infusion. A controlled study. *Acta Endocrinol (Copenh).* 1987 Mar;114(3):433-9.

Hettiarachchi M, Watkinson A, Jenkins AB, Theos V, Ho KK, Kraegen EW. (1996) Growth hormone-induced insulin resistance and its relationship to lipid availability in the rat. *Diabetes.* 1996 Apr;45(4):415-21.

Hindmarsh P, Di Silvio L, Pringle PJ, Kurtz AB, Brook CG. (1988a) Changes in serum insulin concentration during puberty and their relationship to growth hormone. *Clin Endocrinol (Oxf).* 1988 Apr;28(4):381-8.

Hindmarsh PC, Matthews DR, Brook CG. (1988b) Growth hormone secretion in children determined by time series analysis. *Clin Endocrinol (Oxf).* 1988 Jul;29(1):35-44.

Hindmarsh PC, Matthews DR, Brain CE, Pringle PJ, di Silvio L, Kurtz AB, Brook CG. (1989) The half-life of exogenous growth hormone after suppression of endogenous growth hormone secretion with somatostatin. *Clin Endocrinol (Oxf).* 1989 Apr;30(4):443-50.

Hindmarsh PC, Matthews DR, Brain C, Pringle PJ, Brook CG. (1990) The application of deconvolution analysis to elucidate the pulsatile nature of growth hormone secretion using a variable half-life of growth hormone. *Clin Endocrinol (Oxf).* 1990 Jun;32(6):739-47.

Hindmarsh PC, Brain CE, Robinson IC, Matthews DR, Brook CG. (1991) The interaction of growth hormone releasing hormone and somatostatin in the generation of a GH pulse in man. *Clin Endocrinol (Oxf).* 1991 Oct;35(4):353-60.

Ho KY, Veldhuis JD, Johnson ML, Furlanetto R, Evans WS, Alberti KG, Thorner MO. (1988) Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *J Clin Invest.* 1988 Apr;81(4):968-75.

Ho PJ, Friberg RD, Barkan AL. (1992) Regulation of pulsatile growth hormone secretion by fasting in normal subjects and patients with acromegaly. *J Clin Endocrinol Metab.* 1992 Sep;75(3):812-9.

- Ho PJ, Kletter GB, Hopwood NJ, DeMott Friberg R, Barkan AL. (1993)** Somatostatin withdrawal alone is an ineffective generator of pulsatile growth hormone release in man. *Acta Endocrinol (Copenh)*. 1993 Nov;129(5):414-8.
- Hochberg Z, Youdim MB, Amit T. (1994)** A mathematical model for appraisal of the impact of GH binding protein on GH receptor binding. *Growth Regul*. 1994 Mar;4(1):8-13.
- Hoffman RP, Vicini P, Sivitz WI, Cobelli C. (2000)** Pubertal adolescent male-female differences in insulin sensitivity and glucose effectiveness determined by the one compartment minimal model. *Pediatr Res*. 2000 Sep;48(3):384-8.
- Hoeffler JP, Hicks SA, Frawley LS. (1987)** Existence of somatotrope subpopulations which are differentially responsive to insulin-like growth factor I and somatostatin. *Endocrinology*. 1987 May;120(5):1936-41.
- Holl RW, Hartman ML, Veldhuis JD, Taylor WM, Thorner MO. (1991)** Thirty-second sampling of plasma growth hormone in man: correlation with sleep stages. *J Clin Endocrinol Metab*. 1991 Apr;72(4):854-61.
- Holly JM, Biddlecombe RA, Dunger DB, Edge JA, Amiel SA, Howell R, Chard T, Rees LH, Wass JA. (1988)** Circadian variation of GH-independent IGF-binding protein in diabetes mellitus and its relationship to insulin. A new role for insulin? *Clin Endocrinol (Oxf)*. 1988 Dec;29(6):667-75.
- Honeyman TW, Goodman HM. (1980)** Effects of growth hormone on glycogen metabolism in adipose tissue of hypophysectomized rats. *Am J Physiol*. 1980 Apr;238(4):E389-94.
- Hopwood NJ, Forsman PJ, Kenny FM, Drash AL. (1975)** Hypoglycemia in hypopituitary children. *Am J Dis Child*. 1975 Aug;129(8):918-26.
- Horner JM, Hintz RL, Kemp SF. (1981)** Growth hormone and somatomedin in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*. 1981 Dec;53(6):1148-53.
- Hourd P, Edge JA, Dunger DB, Dalton N, Edwards R. (1991)** Urinary growth hormone excretion during puberty in type 1 (insulin-dependent) diabetes mellitus. *Diabet Med*. 1991 Apr;8(3):237-42.
- Ikeda T, Yoshida T, Ito Y, Murakami I, Mokuda O, Tominaga M, Mashiba H. (1987)** Effect of beta-hydroxybutyrate and acetoacetate on insulin and glucagon secretion from perfused rat pancreas. *Arch Biochem Biophys*. 1987 Aug 15;257(1):140-3.

Imaki T, Shibasaki T, Shizume K, Masuda A, Hotta M, Kiyosawa Y, Jibiki K, Demura H, Tsushima T, Ling N. (1985) The effect of free fatty acids on growth hormone (GH)-releasing hormone-mediated GH secretion in man. *J Clin Endocrinol Metab.* 1985 Feb;60(2):290-3.

Imaki T, Shibasaki T, Masuda A, Hotta M, Yamauchi N, Demura H, Shizume K, Wakabayashi I, Ling N. (1986) The effect of glucose and free fatty acids on growth hormone (GH)-releasing factor-mediated GH secretion in rats. *Endocrinology.* 1986 Jun;118(6):2390-4.

Isaacs RE, Gardner DG, Baxter JD. (1987) Insulin regulation of rat growth hormone gene expression. *Endocrinology.* 1987 May;120(5):2022-8.

Iranmanesh A, Grisso B, Veldhuis JD. (1994) Low basal and persistent pulsatile growth hormone secretion are revealed in normal and hypsomatotrophic men studied with a new ultrasensitive chemiluminescence assay. *J Clin Endocrinol Metab.* 1994 Mar;78(3):526-35.

Isales CM, Min L, Hoffman WH. (1999) Acetoacetate and beta-hydroxybutyrate differentially regulate endothelin-1 and vascular endothelial growth factor in mouse brain microvascular endothelial cells. *J Diabetes Complications.* 1999 Mar-Apr;13(2):91-7.

Ismail IS, Scanlon MF, Peters JR. (1991) Effects of ambient glycaemic state on growth hormone responsiveness in insulin-dependent diabetes. *Clin Endocrinol (Oxf).* 1991 Dec;35(6):499-504.

Ismail IS, Scanlon MF, Peters JR. (1993) Cholinergic control of growth hormone (GH) responses to GH-releasing hormone in insulin dependent diabetics: evidence for attenuated hypothalamic somatostatinergic tone and decreased GH autofeedback. *Clin Endocrinol (Oxf).* 1993 Feb;38(2):149-57.

Ismail I, Lewis M, Peters JR, Scanlon MF. (1995) Hypothalamic mediation of reduced GH secretion in diabetic rats: evidence for reduced cholinergic inhibition of somatostatin release. *J Neuroendocrinol.* 1995 Apr;7(4):311-8.

Jaffe CA, Friberg RD, Barkan AL. (1993) Suppression of growth hormone (GH) secretion by a selective GH-releasing hormone (GHRH) antagonist. Direct evidence for involvement of endogenous GHRH in the generation of GH pulses. *J Clin Invest.* 1993 Aug;92(2):695-701.

Jain SK, McVie R, Jackson R, Levine SN, Lim G. (1999) Effect of hyperketonemia on plasma lipid peroxidation levels in diabetic patients. *Diabetes Care.* 1999 Jul;22(7):1171-5.

Jeffcoate SL. (1993) Analytical and clinical significance of peptide hormone heterogeneity with particular reference to growth hormone and luteinizing hormone in serum. *Clin Endocrinol (Oxf).* 1993 Feb;38(2):113-21.

Johnson ML. (1983) Evaluation and propagation of confidence intervals in non linear, asymmetrical variance spaces. Analysis of ligand-binding data. *Biophys J.* 1983 Oct;44(1):101-6.

Johnston DG, Davies RR, Prescott RW. (1985) Regulation of growth hormone secretion in man: a review. *J R Soc Med.* 1985 Apr;78(4):319-27. Review

Jones PR, Wilkinson S, Davies PS. (1985) A revision of body surface area estimations. *Eur J Appl Physiol Occup Physiol.* 1985;53(4):376-9.

Jorgensen JO, Flyvbjerg A, Christiansen JS. (1989) The metabolic clearance rate, serum half-time and apparent distribution space of authentic biosynthetic human growth hormone in growth hormone-deficient patients. *Acta Endocrinol (Copenh).* 1989 Jan;120(1):8-13.

Jorgensen JO, Moller N, Lauritzen T, Christiansen JS. (1990) Pulsatile versus continuous intravenous administration of growth hormone (GH) in GH-deficient patients: effects on circulating insulin-like growth factor-I and metabolic indices. *J Clin Endocrinol Metab.* 1990 Jun;70(6):1616-23.

Keenan DM, Veldhuis JD, Yang R. (1998) Joint recovery of pulsatile and basal hormone secretion by stochastic non linear random-effects analysis. *Am J Physiol.* 1998 Dec;275(6 Pt 2):R1939-49.

Keller U, Schnell H, Girard J, Stauffacher W. (1984) Effect of physiological elevation of plasma growth hormone levels on ketone body kinetics and lipolysis in normal and acutely insulin-deficient man. *Diabetologia.* 1984 Feb;26(2):103-8.

Kim JK, Choi CS, Youn JH. (1999) Acute effect of growth hormone to induce peripheral insulin resistance is independent of FFA and insulin levels in rats. *Am J Physiol.* 1999 Oct;277(4 Pt 1):E742-9.

Kerchner J, Colaluca DM, Juhl RP. (1980) Effect of whole blood on insulin adsorption onto intravenous infusion systems. *Am J Hosp Pharm.* 1980 Oct;37(10):1323-5.

Kobayashi K, Amemiya S, Sawanobori E, Higashida K, Ishihara T, Kobayashi K, Kato K, Nakazawa S. (1997) Role of IGF binding protein-1 in the dawn phenomenon and glycemic control in children and adolescents with IDDM. *Diabetes Care.* 1997 Sep;20(9):1442-7.

Koch BD, Schonbrunn A. (1984) The somatostatin receptor is directly coupled to adenylate cyclase in GH4C1 pituitary cell membranes. *Endocrinology.* 1984 May;114(5):1784-90.

Kollind M, Moberg E, Lins PE, Adamson U. (1990) Exogenous somatostatin raises plasma insulin levels in patients with insulin-dependent diabetes mellitus. *Horm Metab Res.* 1990 Nov;22(11):581-3.

Komjati M, Bratusch-Marrain P, Waldhausl W. (1986) Superior efficacy of pulsatile versus continuous hormone exposure on hepatic glucose production in vitro. *Endocrinology*. 1986 Jan;118(1):312-9.

Kopelman PG, Mason AC, Noonan K, Monson JP. (1988) Growth hormone response to growth hormone releasing factor in diabetic men. *Clin Endocrinol (Oxf)*. 1988 Jan;28(1):33-8.

Kowarski A, Thompson RG, Migeon CJ, Blizzard RM. (1971) Determination of integrated plasma concentrations and true secretion rates of human growth hormone. *J Clin Endocrinol Metab*. 1971 Mar;32(3):356-60.

Krulich L, Dhariwal AP, McCann SM. (1968) Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. *Endocrinology*. 1968 Oct;83(4):783-90.

Kuilboer MM, Kleijer B, Kamp GA, Veldhuis JD, Johnson ML, Wit JM. (1992) Comparison of four computerized models to estimate 24-hour growth hormone secretion in girls with Turner's syndrome. *Horm Res*. 1992;38(3-4):125-33.

Kuku SF, Jaspán JB, Emmanouel DS, Zeidler A, Katz AI, Rubenstein AH. (1976) Heterogeneity of plasma glucagon. Circulating components in normal subjects and patients with chronic renal failure. *J Clin Invest*. 1976 Sep;58(3):742-50.

Landau D, Domene H, Flyvbjerg A, Gronbaek H, Roberts CT Jr, Argov S, LeRoith D. (1998) Differential expression of renal growth hormone receptor and its binding protein in experimental diabetes mellitus. *Growth Horm IGF Res*. 1998 Feb;8(1):39-45.

Lanes R, Recker B, Fort P, Lifshitz F. (1985) Impaired somatomedin generation test in children with insulin-dependent diabetes mellitus. *Diabetes*. 1985 Feb;34(2):156-60.

Lang DA, Matthews DR, Peto J, Turner RC. (1979) Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. *N Engl J Med*. 1979 Nov 8;301(19):1023-7.

Langendonk JG, Meinders AE, Burggraaf J, Frolich M, Roelen CA, Schoemaker RC, Cohen AF, Pijl H. (1999) Influence of obesity and body fat distribution on growth hormone kinetics in humans. *Am J Physiol*. 1999 Nov;277(5 Pt 1):E824-9.

Langendonk JG, Veldhuis JD, Burggraaf J, Schoemaker RC, Cohen AF, EdoMeinders A, Pijl H. (2000) Estimation of growth hormone secretion rate: impact of kinetic assumptions intrinsic to the analytical approach. *Am J Physiol Regul Integr Comp Physiol*. 2001 Jan;280(1):R225-32

Lanzi R, Andreotti AC, Caumo A, Manzoni MF, Losa M, Malighetti ME, Pontiroli AE. (1995) Assessment of growth hormone (GH) plasma clearance rate, half-life, and volume of distribution in acromegalic patients: the combined GH-octreotide infusion. *J Clin Endocrinol Metab.* 1995 Nov;80(11):3279-83.

Laron Z, Mannheimer S, Guttman S. (1965) Plasma disappearance of various ¹³¹I-labelled growth hormone preparations in man and rabbit. *Nature.* 1965 Jul 17;207(994):298-9.

Laughlin GA, Yen SS. (1996) Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. *J Clin Endocrinol Metab.* 1996 Dec;81(12):4301-9.

Laursen T, Jorgensen JO, Jakobsen G, Hansen BL, Christiansen JS. (1995) Continuous infusion versus daily injections of growth hormone (GH) for 4 weeks in GH-deficient patients. *J Clin Endocrinol Metab.* 1995 Aug;80(8):2410-8.

Laville M, Rigalleau V, Riou JP, Beylot M. (1995) Respective role of plasma nonesterified fatty acid oxidation and total lipid oxidation in lipid-induced insulin resistance. *Metabolism.* 1995 May;44(5):639-44.

Leung KC, Ho KK. (1997) Stimulation of mitochondrial fatty acid oxidation by growth hormone in human fibroblasts. *J Clin Endocrinol Metab.* 1997 Dec;82(12):4208-13.

Lewis LD, Williams JA. (1987) Structural characterization of the somatostatin receptor in rat anterior pituitary membranes. *Endocrinology.* 1987 Aug;121(2):486-92.

Lewis UJ, Lewis LJ, Salem MA, Staten NR, Galosy SS, Krivi GG. (1991) A recombinant-DNA-derived modification of human growth hormone (hGH44-191) with enhanced diabetogenic activity. *Mol Cell Endocrinol.* 1991 Jun;78(1-2):45-54.

Lins PE, Efendic S. (1976) Hyperglycemia induced by somatostatin in normal subjects. *Horm Metab Res.* 1976 Nov;8(6):497-8.

Lipman RL, Taylor AL, Conly P, Mintz DH. (1972) Metabolic clearance rate of growth hormone in juvenile diabetes mellitus. *Diabetes.* 1972 Mar;21(3):175-7.

Locatelli V, Torsello A, Redaelli M, Ghigo E, Massare F, Muller EE. (1986) Cholinergic agonist and antagonist drugs modulate the growth hormone response to growth hormone-releasing hormone in the rat: evidence for mediation by somatostatin. *J Endocrinol.* 1986 Nov;111(2):271-8.

Lussier BT, French MB, Moor BC, Kraicer J. (1991) Free intracellular Ca^{2+} concentration and growth hormone (GH) release from purified rat somatotrophs. III. Mechanism of action of GH-releasing factor and somatostatin. *Endocrinology*. 1991 Jan;128(1):592-603.

Luthman M, Jonsdottir I, Skoog B, Wivall IL, Roos P, Werner S. (1990) Monoclonal antibodies reveal circulating growth hormone of high molecular weight not detectable by conventional assays. *Acta Endocrinol (Copenh)*. 1990 Sep;123(3):317-25.

Maccario M, Aimaretti G, Corneli G, Gauna C, Grottoli S, Bidlingmaier M, Strasburger CJ, Dieguez C, Casanueva FF, Ghigo E. (2000) Short-term fasting abolishes the sex-related difference in GH and leptin secretion in humans. *Am J Physiol Endocrinol Metab*. 2000 Aug;279(2):E411-6.

MacFarlane IA, Stafford S, Wright AD. Circulating growth hormone forms in type 1 diabetic subjects: comparison with normal subjects and acromegalics. *Acta Endocrinol (Copenh)*. 1986 Aug;112(4):547-51.

MacGillivray MH, Frohman LA, Doe J. (1970) Metabolic clearance and production rates of human growth hormone in subjects with normal and abnormal growth. *J Clin Endocrinol Metab*. 1970 May;30(5):632-8.

MacGorman LR, Rizza RA, Gerich JE. (1981) Physiological concentrations of growth hormone exert insulin-like and insulin antagonistic effects on both hepatic and extrahepatic tissues in man. *J Clin Endocrinol Metab*. 1981 Sep;53(3):556-9.

Maes M, Ketelslegers JM, Underwood LE. (1983) Low plasma somatomedin-C in streptozotocin-induced diabetes mellitus. Correlation with changes in somatogenic and lactogenic liver binding sites. *Diabetes*. 1983 Nov;32(11):1060-9.

Maiter D, Underwood LE, Maes M, Davenport ML, Ketelslegers JM. (1988) Different effects of intermittent and continuous growth hormone (GH) administration on serum somatomedin-C/insulin-like growth factor I and liver GH receptors in hypophysectomized rats. *Endocrinology*. 1988 Aug;123(2):1053-9.

Maiter D, Walker JL, Adam E, Moatsstaats B, Mulumba N, Ketelslegers JM, Underwood LE. (1982) Differential regulation by growth hormone (GH) of insulin-like growth factor I and GH receptor/binding protein gene expression in rat liver. *Endocrinology*. 1992 Jun;130(6):3257-64.

Marshall S, Olefsky JM. (1983) Separate intracellular pathways for insulin receptor recycling and insulin degradation in isolated rat adipocytes. *J Cell Physiol*. 1983 Nov;117(2):195-203.

Martina V, Tagliabue M, Maccario M, Bertagna A, Ghigo E, Massara F, Camanni F (1987) Pirenzepine blunts the nocturnal growth hormone release in insulin dependent diabetes. *Horm Metab Res.* 1987 Sep;19(9):449-50.

Martina V, Bruno G, Tagliabue M, Maccario M, Bertagna S, Zumpano E, Arvat E, Ghigo E, Camanni F. (1997) Repeated administration of growth hormone-releasing hormone with or without previous administration of pyridostigmine in insulin-dependent diabetes mellitus. *Horm Metab Res.* 1997 Apr;29(4):180-3.

Massara F, Ghigo E, Molinatti P, Mazza E, Locatelli V, Muller EE, Camanni F. (1986) Potentiation of cholinergic tone by pyridostigmine bromide re-instates and potentiates the growth hormone responsiveness to intermittent administration of growth hormone-releasing factor in man. *Acta Endocrinol (Copenh).* 1986 Sep;113(1):12-6.

Masuda A, Shibasaki T, Nakahara M, Imaki T, Kiyosawa Y, Jibiki K, Demura H, Shizume K, Ling N. (1985) The effect of glucose on growth hormone (GH)-releasing hormone-mediated GH secretion in man. *J Clin Endocrinol Metab.* 1985 Mar;60(3):523-6.

Matis JH, Wehrly TE, Gerald KB. (1985) Use of residence time moments in compartmental analysis. *Am J Physiol.* 1985 Oct;249(4 Pt 1):E409-15.

Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC. (1983a) Pulsatile insulin has greater hypoglycemic effect than continuous delivery. *Diabetes.* 1983 Jul;32(7):617-21.

Matthews DR, Lang DA, Burnett MA, Turner RC. (1983b) Control of pulsatile insulin secretion in man. *Diabetologia.* 1983 Apr;24(4):231-7.

Matthews DR, Rudenski AS, Burnett MA, Darling P, Turner RC. (1985) The half-life of endogenous insulin and C-peptide in man assessed by somatostatin suppression. *Clin Endocrinol (Oxf).* 1985 Jul;23(1):71-9.

Matthews DR. (1988) Time series analysis in endocrinology. *Acta Paediatr Scand Suppl.* 1988;347:55-62.

Matthews DR, Hosker JP. (1989) Unbiased and flexible iterative computer program to achieve glucose clamping. *Diabetes Care.* 1989 Feb;12(2):156-9.

Matthews DR, Edge JA, Dunger DB. (1990) An unbiased glucose clamp method using a variable insulin infusion: its application in diabetic adolescents. *Diabet Med.* 1990 Mar-Apr;7(3):246-51.

Matthews DR, Hindmarsh PC, Pringle PJ, Brook CG. (1991) A distribution method for analysing the baseline of pulsatile endocrine signals as exemplified by 24-hour growth hormone profiles. *Clin Endocrinol (Oxf).* 1991 Sep;35(3):245-52.

Matyka KA, Crowne EC, Havel PJ, Macdonald IA, Matthews D, Dunger DB. (1999) Counterregulation during spontaneous nocturnal hypoglycemia in prepubertal children with type 1 diabetes. *Diabetes Care*. 1999 Jul;22(7):1144-50.

Mayo KE, Miller T, DeAlmeida V, Godfrey P, Zheng J, Cunha SR. (2000) Regulation of the pituitary somatotroph cell by GHRH and its receptor. *Recent Prog Horm Res*. 2000;55:237-66; discussion 266-7. Review

Mazza E, Ghigo E, Boffano G, Valetto M, Maccario M, Arvat E, Bellone J, Procopio M, Muller EE, Camanni F. (1994) Effects of direct and indirect acetylcholine receptor agonists on growth hormone secretion in humans. *Eur J Pharmacol*. 1994 Mar 11;254(1-2):17-20.

McGarry JD, Foster DW. (1979) Hormonal control of ketogenesis. *Adv Exp Med Biol*. 1979;111:79-96. Review.

McGuire EA, Helderman JH, Tobin JD, Andres R, Berman M. (1976) Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol*. 1976 Oct;41(4):565-73.

McMahon M, Gerich J, Rizza R. (1988) Effects of glucocorticoids on carbohydrate metabolism. *Diabetes Metab Rev*. 1988 Feb;4(1):17-30. Review.

Melmed S. (1984) Insulin suppresses growth hormone secretion by rat pituitary cells. *J Clin Invest*. 1984 May;73(5):1425-33.

Melmed S, Yamashita S. (1986) Insulin-like growth factor-I action on hypothyroid rat pituitary cells: suppression of triiodothyronine-induced growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology*. 1986 Apr;118(4):1483-90.

Menon RK, Arslanian S, May B, Cutfield WS, Sperling MA. (1992) Diminished growth hormone-binding protein in children with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*. 1992 Apr;74(4):934-8.

Mercado M, Molitch ME, Baumann G. (1992) Low plasma growth hormone binding protein in IDDM. *Diabetes*. 1992 May;41(5):605-9.

Merriam GR, Wachter KW. (1982) Algorithms for the study of episodic hormone secretion. *Am J Physiol*. 1982 Oct;243(4):E310-8.

Metcalfe P, Johnston DG, Nosadini R, Orskov H, Alberti KG. (1981) Metabolic effects of acute and prolonged growth hormone excess in normal and insulin-deficient man. *Diabetologia*. 1981 Feb;20(2):123-8.

Miller JD, Wright NM, Lester SE, Felsing NE, Linzer J, Chan E, White DA, Charles MA. (1992) Spontaneous and stimulated growth hormone release in adolescents with type I diabetes mellitus: effects of metabolic control. *J Clin Endocrinol Metab*. 1992 Oct;75(4):1087-91.

Miller TL, Godfrey PA, Dealmeida VI, Mayo KE. (1999) The rat growth hormone-releasing hormone receptor gene: structure, regulation, and generation of receptor isoforms with different signaling properties. *Endocrinology*. 1999 Sep;140(9):4152-65.

Milner RD. (1985) Growth hormone 1985. *Br Med J (Clin Res Ed)*. 1985 Dec 7;291(6509):1593-4.

Mogensen CE. (1990) Prediction of clinical diabetic nephropathy in IDDM patients. Alternatives to microalbuminuria? *Diabetes*. 1990 Jul;39(7):761-7. Review.

Moller N, Jorgensen JO, Moller J, Bak JF, Porksen N, Alberti KG, Schmitz O. (1990) Substrate metabolism during modest hyperinsulinemia in response to isolated hyperketonemia in insulin-dependent diabetic subjects. *Metabolism*. 1990 Dec;39(12):1309-13.

Moller N, Jorgensen JO, Abildgaard N, Orskov L, Schmitz O, Christiansen JS. (1991) Effects of growth hormone on glucose metabolism. *Horm Res*. 1991;36 Suppl 1:32-5. Review.

Moller N, Schmitz O, Porksen N, Moller J, Jorgensen JO. (1992a) Dose-response studies on the metabolic effects of a growth hormone pulse in humans. *Metabolism*. 1992 Feb;41(2):172-5.

Moller N, Schmitz O, Moller J, Butler PC. (1992b) Effects of a physiological growth hormone pulse on substrate metabolism in insulin-dependent (type 1) diabetic subjects. *J Clin Endocrinol Metab*. 1992 Aug;75(2):432-6.

Moller N, Bagger JP, Schmitz O, Jorgensen JO, Ovesen P, Moller J, Alberti KG, Orskov H (1995) Somatostatin enhances insulin-stimulated glucose uptake in the perfused human forearm. *J Clin Endocrinol Metab*. 1995 Jun;80(6):1789-93.

Molnar GD, Taylor WF, Langworthy A, Fatourehchi V. (1972) Diurnal growth hormone and glucose abnormalities in unstable diabetics: studies of ambulatory-fed subjects during continuous blood glucose analysis. *J Clin Endocrinol Metab*. 1972 May;34(5):837-46.

Moran A, Jacobs DR Jr, Steinberger J, Hong CP, Prineas R, Luepker R, Sinaiko AR. (1999) Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes*. 1999 Oct;48(10):2039-44.

Moreau JP, DeFeudis FV. (1987) Pharmacological studies of somatostatin and somatostatin-analogues: therapeutic advances and perspectives. *Life Sci*. 1987 Feb 2;40(5):419-37. Review.

Morgan CR, Lazarow A. (1965) Immunoassay of pancreatic and plasma insulin following alloxan injection of rats. *Diabetes*. 1965 Oct;14(10):669-71.

Muller EE, Rolla M, Ghigo E, Belliti D, Arvat E, Andreoni A, Torsello A, Locatelli V, Camanni F. (1995) Involvement of brain catecholamines and acetylcholine in growth hormone hypersecretory states. Pathophysiological, diagnostic and therapeutic implications. *Drugs*. 1995 Nov;50(5):805-37.

Review.

Muller EE, Rolla M. (1996) Aspects of the neuroendocrine control of somatotrophic function in calorically restricted dogs and patients with eating disorders: studies with cholinergic drugs. *Psychiatry Res*. 1996 Apr 16;62(1):51-63.

Muller AF, Janssen JA, Hofland LJ, Lamberts SW, Bidlingmaier M, Strasburger CJ, van der Lely AJ. (2001) Blockade of the growth hormone (GH) receptor unmasks rapid GH-releasing peptide-6-mediated tissue-specific insulin resistance. *J Clin Endocrinol Metab*. 2001 Feb;86(2):590-3.

Mullis P, Volkle H, Sigrist V, Zuppinger K. (1988) Comparison of fructosamine and glycated haemoglobin in children with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*. 1988 Jun;31(6):351-4.

Murdoch AP, Diggle PJ, Dunlop W, Kendall-Taylor P. (1985) Determination of the frequency of pulsatile luteinizing hormone secretion by time series analysis. *Clin Endocrinol (Oxf)*. 1985 Mar;22(3):341-6.

Murphy LJ, Vrhovsek E, Sutherland RL, Lazarus L. (1984) Growth hormone binding to cultured human breast cancer cells. *J Clin Endocrinol Metab*. 1984 Jan;58(1):149-56.

Nass R, Toogood AA, Hellmann P, Bissonette E, Gaylinn B, Clark R, Thorner MO. (2000) Intracerebroventricular administration of the rat growth hormone (GH) receptor antagonist G118R stimulates GH secretion: evidence for the existence of short loop negative feedback of GH. *J Neuroendocrinol*. 2000 Dec;12(12):1194-9.

Navalesi R, Pilo A, Vigneri R. (1975) Growth hormone kinetics in diabetic patients. *Diabetes*. 1975 Apr;24(4):317-27.

Navalesi R, Pilo A, Ferrannini E. (1978) Kinetic analysis of plasma insulin disappearance in nonketotic diabetic patients and in normal subjects. A tracer study with 125I-insulin. *J Clin Invest*. 1978 Jan;61(1):197-208.

Ndon JA, Giustina A, Wehrenberg WB. (1984) Hypothalamic regulation of impaired growth hormone secretion in diabetic rats. 2. Studies in spontaneously diabetic BB Worcester rats. *Neuroendocrinology*. 1992 May;55(5):506-11.

Nielsen MF, Dinneen S, Basu A, Basu R, Alzaid A, Rizza RR. (1998) Failure of nocturnal changes in growth hormone to alter carbohydrate tolerance the following morning. *Diabetologia*. 1998 Sep;41(9):1064-72.

- Nielsen S, Moller N, Christiansen JS, Jorgensen JO. (2001) Pharmacological antilipolysis restores insulin sensitivity during growth hormone exposure. *Diabetes*. 2001 Oct;50(10):2301-8.
- Nieves-Rivera F, Rogol AD, Veldhuis JD, Branscom DK, Martha PM Jr, Clarke WL. (1993) Alterations in growth hormone secretion and clearance in adolescent boys with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*. 1993 Sep;77(3):638-43.
- Nunez EA. (1997) Fatty acids involved in signal cross-talk between cell membrane and nucleus. *Prostaglandins Leukot Essent Fatty Acids*. 1997 Oct;57(4-5):429-34. Review.
- Oerter KE, Guardabasso V, Rodbard D. (1986) Detection and characterization of peaks and estimation of instantaneous secretory rate for episodic pulsatile hormone secretion. *Comput Biomed Res*. 1986 Apr;19(2):170-91.
- Okuda Y, Kawai K, Ohmori H, Yamashita K. (1991) Ketone body utilization and its metabolic effect in resting muscles of normal and streptozotocin-diabetic rats. *Endocrinol Jpn*. 1991 Jun;38(3):245-51.
- Okuda Y, Pena J, Chou J, Field JB. (2001) Acute effects of growth hormone on metabolism of pancreatic hormones, glucose and ketone bodies. *Diabetes Res Clin Pract*. 2001 Jul;53(1):1-8.
- Olchovsky D, Bruno JF, Wood TL, Gelato MC, Leidy JW Jr, Gilbert JM Jr, Berelowitz M. (1990) Altered pituitary growth hormone (GH) regulation in streptozotocin-diabetic rats: a combined defect of hypothalamic somatostatin and GH-releasing factor. *Endocrinology*. 1990 Jan;126(1):53-61.
- Olefsky JM, Revers RR, Prince M, Henry RR, Garvey WT, Scarlett JA, Kolterman OG. (1985) Insulin resistance in non-insulin dependent (type II) and insulin dependent (type I) diabetes mellitus. *Adv Exp Med Biol*. 1985;189:176-205.
- Olsen BS, Johannesen J, Sjolie AK, Borch-Johnsen K, Hougaard P, Thorsteinsson B, Pramming S, Marinelli K, Mortensen HB. (1999) Metabolic control and prevalence of microvascular complications in young Danish patients with Type 1 diabetes mellitus. Danish Study Group of Diabetes in Childhood. *Diabet Med*. 1999 Jan;16(1):79-85.
- Orskov H. (1985) Growth hormone hyperproduction inducing some of the vicious circles in diabetes mellitus. *Acta Med Scand*. 1985;217(4):343-6.
- Orskov H. (1996) Somatostatin, growth hormone, insulin-like growth factor-1, and diabetes: friends or foes? *Metabolism*. 1996 Aug;45(8 Suppl 1):91-5.

Orskov L, Schmitz O, Jorgensen JO, Arnfred J, Abildgaard N, Christiansen JS, Alberti KG, Orskov H. (1989) Influence of growth hormone on glucose-induced glucose uptake in normal men as assessed by the hyperglycemic clamp technique. *J Clin Endocrinol Metab.* 1989 Feb;68(2):276-82.

Ovesen P, Vahl N, Fisker S, Veldhuis JD, Christiansen JS, Jorgensen JO. (1998) Increased pulsatile, but not basal, growth hormone secretion rates and plasma insulin-like growth factor I levels during the periovulatory interval in normal women. *J Clin Endocrinol Metab.* 1998 May;83(5):1662-7. PMID: 9589674; UI: 9825158

Owens D, Srivastava MC, Tompkins CV, Nabarro JD, Sonksen PH. (1973) Studies on the metabolic clearance rate, apparent distribution space and plasma half-disappearance time of unlabelled human growth hormone in normal subjects and in patients with liver disease, renal disease, thyroid disease and diabetes mellitus. *Eur J Clin Invest.* 1973 Jul;3(4):284-94.

Owens D, Srivastava MC, Tompkins CV, Nabarro JD, Sonksen PH. (1973) Studies on the metabolic clearance rate, apparent distribution space and plasma half-disappearance time of unlabelled human growth hormone in normal subjects and in patients with liver disease, renal disease, thyroid disease and diabetes mellitus. *Eur J Clin Invest.* 1973 Jul;3(4):284-94.

Pacini G, Finegood DT, Bergman RN. (1982) A minimal-model-based glucose clamp yielding insulin sensitivity independent of glycemia. *Diabetes.* 1982 May;31(5 Pt 1):432-41.

Page MD, Koppeschaar HP, Dieguez C, Gibbs JT, Hall R, Peters JR, Scanlon MF. (1987a) Cholinergic muscarinic receptor blockade with pirenzepine abolishes slow wave sleep-related growth hormone release in young patients with insulin-dependent diabetes mellitus. *Clin Endocrinol (Oxf).* 1987 Mar;26(3):355-9.

Page MD, Koppeschaar HP, Edwards CA, Dieguez C, Scanlon MF. (1987b) Additive effects of growth hormone releasing factor and insulin hypoglycaemia on growth hormone release in man. *Clin Endocrinol (Oxf).* 1987 May;26(5):589-95.

Painson JC, Tannenbaum GS. (1983) Effects of intracellular glucopenia on pulsatile growth hormone secretion: mediation in part by somatostatin. *Endocrinology.* 1985 Sep;117(3):1132-8.

Pan JW, Rothman TL, Behar KL, Stein DT, Hetherington HP. (2000) Human brain beta-hydroxybutyrate and lactate increase in fasting-induced ketosis. *J Cereb Blood Flow Metab.* 2000 Oct;20(10):1502-7.

Panzeri G, Torsello A, Cella SG, Muller EE, Locatelli V. (1990) Age-related modulatory activity by a cholinergic agonist on the growth hormone response to GH-releasing hormone in the rat. *Proc Soc Exp Biol Med.* 1990 Apr;193(4):301-5.

Paolisso G, Scheen AJ, Verdin EM, Luyckx AS, Lefebvre PJ. (1986) Insulin oscillations per se do not affect glucose turnover parameters in normal man. *J Clin Endocrinol Metab.* 1986 Aug;63(2):520-5.

Paolisso G, Sgambato S, Passariello N, Scheen A, D'Onofrio F, Lefebvre PJ. (1987) Greater efficacy of pulsatile insulin in type I diabetics critically depends on plasma glucagon levels. *Diabetes.* 1987 May;36(5):566-70.

Parving HH. (1975) Microvascular permeability to plasma proteins in hypertension and diabetes mellitus in man—on the pathogenesis of hypertensive and diabetic microangiopathy. *Dan Med Bull.* 1975 Sep;22(6):217-33.

Patti ME. (1999) Nutrient modulation of cellular insulin action. *Ann N Y Acad Sci.* 1999 Nov 18;892:187-203. Review.

Pau KY, Spies HG. (1997) Neuroendocrine signals in the regulation of gonadotropin-releasing hormone secretion. *Chin J Physiol.* 1997 Dec 31;40(4):181-96. Review.

Penalva A, Burguera B, Casabiell X, Tresguerres JA, Dieguez C, Casanueva FF. (1989) Activation of cholinergic neurotransmission by pyridostigmine reverses the inhibitory effect of hyperglycemia on growth hormone (GH) releasing hormone-induced GH secretion in man: does acute hyperglycemia act through hypothalamic release of somatostatin? *Neuroendocrinology.* 1989 May;49(5):551-4.

Penalva A, Muruais C, Casanueva FF, Dieguez C. (1990) Effect of enhancement of endogenous cholinergic tone with pyridostigmine on the dose-response relationships of growth hormone (GH)-releasing hormone-induced GH secretion in normal subjects. *J Clin Endocrinol Metab.* 1990 Feb;70(2):324-7.

Peng XD, Park S, Gadelha MR, Coschigano KT, Kopchick JJ, Frohman LA, Kineman RD. (2001) The growth hormone (GH)-axis of GH receptor/binding protein gene-disrupted and metallothionein-human GH-releasing hormone transgenic mice: hypothalamic neuropeptide and pituitary receptor expression in the absence and presence of GH feedback. *Endocrinology.* 2001 Mar;142(3):1117-23.

Perriello G, De Feo P, Bolli GB. (1988) The dawn phenomenon: nocturnal blood glucose homeostasis in insulin-dependent diabetes mellitus. *Diabet Med.* 1988 Jan;5(1):13-21. Review.

Perriello G, De Feo P, Torlone E, Fanelli C, Santeusano F, Brunetti P, Bolli GB. (1990) Nocturnal spikes of growth hormone secretion cause the dawn phenomenon in type 1 (insulin-dependent) diabetes mellitus by decreasing hepatic (and extrahepatic) sensitivity to insulin in the absence of insulin waning. *Diabetologia*. 1990 Jan;33(1):52-9.

Perriello G, De Feo P, Torlone E, Fanelli C, Santeusano F, Brunetti P, Bolli GB. (1991) The dawn phenomenon in type 1 (insulin-dependent) diabetes mellitus: magnitude, frequency, variability, and dependency on glucose counter-regulation and insulin sensitivity. *Diabetologia*. 1991 Jan;34(1):21-8.

Pesce G, Guillaume V, Dutour A, Dadoun F, Joanny P, Steinberg J, Oliver C. (1994) Effect of streptozotocin-induced diabetes on somatostatin receptors in the anterior pituitary, hypothalamus and cerebral cortex of the male rat. *Life Sci*. 1994;54(24):1927-33.

Peters JR, Evans PJ, Page MD, Hall R, Gibbs JT, Dieguez C, Scanlon MF. (1986) Cholinergic muscarinic receptor blockade with pirenzepine abolishes slow wave sleep-related growth hormone release in normal adult males. *Clin Endocrinol (Oxf)*. 1986 Aug;25(2):213-7.

Pfeiffer A, Schatz H. (1995) Diabetic microvascular complications and growth factors. *Exp Clin Endocrinol Diabetes*. 1995;103(1):7-14. Review.

Phillips LS, Harp JB, Goldstein S, Klein J, Pao CI. (1990) Regulation and action of insulin-like growth factors at the cellular level. *Proc Nutr Soc*. 1990 Oct;49(3):451-8. Review.

Piatti PM, Monti LD, Caumo A, Conti M, Magni F, Galli-Kienle M, Fochesato E, Pizzini A, Baldi L, Valsecchi G, Pontiroli AE. (1999) Mediation of the hepatic effects of growth hormone by its lipolytic activity. *J Clin Endocrinol Metab*. 1999 May;84(5):1658-63.

Pietschmann P, Schernthaner G, Luger A. (1986) Effect of cholinergic muscarinic receptor blockade on human growth hormone (GH)-releasing hormone-(1-44)-induced GH secretion in acromegaly and type I diabetes mellitus. *J Clin Endocrinol Metab*. 1986 Aug;63(2):389-93.

Pincus SM, Gevers EF, Robinson IC, van den Berg G, Roelfsema F, Hartman ML, Veldhuis JD. (1996) Females secrete growth hormone with more process irregularity than males in both humans and rats. *Am J Physiol*. 1996 Jan; 270(1 Pt 1)

Plotsky PM, Vale W. (1985) Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. *Science*. 1985 Oct 25;230(4724):461-3.

Pombo M, Pombo CM, Astorga R, Cordido F, Popovic V, Garcia-Mayor RV, Dieguez C, Casanueva FF. (1999) Regulation of growth hormone secretion by signals produced by the adipose tissue. *J Endocrinol Invest.* 1999;22(5 Suppl):22-6. Review.

Prelevic GM. (1997) Insulin resistance in polycystic ovary syndrome. *Curr Opin Obstet Gynecol.* 1997 Jun; 9(3):193-201. Review

Press M, Tamborlane WV, Sherwin RS (1984a) Importance of raised growth hormone levels in mediating the metabolic derangements of diabetes. *N Engl J Med* 1984 Mar 29;310(13):810-5

Press M, Tamborlane WV, Thorner MO, Vale W, Rivier J, Gertner JM, Sherwin RS (1984b) Pituitary response to growth hormone-releasing factor in diabetes. Failure of glucose-mediated suppression. *Diabetes* 1984 Aug;33(8):804-6

Press M, Caprio S, Tamborlane WV, Bhushan R, Thorner M, Vale W, Rivier J, Sherwin RS. (1992) Pituitary response to growth hormone-releasing hormone in IDDM. Abnormal responses to insulin and hyperglycemia. *Diabetes.* 1992 Jan;41(1):17-21.

Quabbe HJ, Bratzke HJ, Siegers U, Elban K. (1972) Studies on the relationship between plasma free fatty acids and growth hormone secretion in man. *J Clin Invest.* 1972 Sep;51(9):2388-98.

Quabbe HJ, Trompke M, Luyckx AS. (1983) Influence of ketone body infusion on plasma growth hormone and glucagon in man. *J Clin Endocrinol Metab.* 1983 Sep;57(3):613-8.

Quabbe HJ. (1985) Hypothalamic control of GH secretion: pathophysiology and clinical implications. *Acta Neurochir (Wien).* 1985;75(1-4):60-71.

Radetti G, Paganini C, Antoniazzi F, Pasquino B, Valentini R, Gentili L, Tato L. (1997) Growth hormone-binding proteins, IGF-I and IGF-binding proteins in children and adolescents with type 1 diabetes mellitus. *Horm Res.* 1997;47(3):110-5.

Randle PJ. (1998) Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev.* 1998 Dec;14(4):263-83. Review.

Refetoff S, Sonksen PH. (1970) Disappearance rate of endogenous and exogenous human growth hormone in man. *J Clin Endocrinol Metab.* 1970 Mar;30(3):386-92.

- Reisine T. (1984)** Somatostatin desensitization: loss of the ability of somatostatin to inhibit cyclic AMP accumulation and adrenocorticotropin hormone release. *J Pharmacol Exp Ther.* 1984 Apr;229(1):14-20.
- Reutens AT, Veldhuis JD, Hoffman DM, Leung KC, Ho KK. (1996)** A highly sensitive growth hormone (GH) enzyme-linked immunosorbent assay uncovers increased contribution of a tonic mode of GH secretion in adults with organic GH deficiency. *J Clin Endocrinol Metab.* 1996 Apr;81(4):1591-7.
- Richardson SB, Hollander CS, D'Eletto R, Greenleaf PW, Thaw C. (1980)** Acetylcholine inhibits the release of somatostatin from rat hypothalamus in vitro. *Endocrinology.* 1980 Jul;107(1):122-9.
- Richelsen B. (1997)** Action of growth hormone in adipose tissue. *Horm Res.* 1997;48 Suppl 5:105-10. Review
- Rigamonti AE, Marazzi N, Cella SG, Cattaneo L, Muller EE. (1998)** Growth hormone responses to growth hormone-releasing hormone and hexarelin in fed and fasted dogs: effect of somatostatin infusion or pretreatment with pirenzepine. *J Endocrinol.* 1998 Feb;156(2):341-8.
- Rizza RA, Cryer PE, Gerich JE. (1979)** Role of glucagon, catecholamines, and growth hormone in human glucose counterregulation. Effects of somatostatin and combined alpha- and beta-adrenergic blockade on plasma glucose recovery and glucose flux rates after insulin-induced hypoglycemia. *J Clin Invest.* 1979 Jul;64(1):62-71.
- Rizza RA, Gerich JE, Haymond MW, Westland RE, Hall LD, Clemens AH, Service FJ. (1980)** Control of blood sugar in insulin-dependent diabetes: comparison of an artificial endocrine pancreas, continuous subcutaneous insulin infusion, and intensified conventional insulin therapy. *N Engl J Med.* 1980 Dec 4;303(23):1313-8.
- Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI. (1996)** Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest.* 1996 Jun 15;97(12):2859-65.
- Ross RJ, Borges F, Grossman A, Smith R, Nghofoong L, Rees LH, Savage MO, Besser GM. (1987)** Growth hormone pretreatment in man blocks the response to growth hormone-releasing hormone; evidence for a direct effect of growth hormone. *Clin Endocrinol (Oxf).* 1987 Jan;26(1):117-23.
- Roupas P, Herington AC. (1987a)** Processing of growth hormone by rat adipocytes in primary culture: differentiation between release of intact hormone and degradative processing. *Endocrinology.* 1987 Oct;121(4):1521-30.

Roupas P, Herington AC. (1987b) Receptor-mediated endocytosis and degradative processing of growth hormone by rat adipocytes in primary culture. *Endocrinology* 1987 May;120(5):2158-65.

Royston JP. (1989) The statistical analysis of pulsatile hormone secretion data. *Clin Endocrinol (Oxf)*. 1989 Feb;30(2):201-10. Review.

Russell-Jones DL, Rattray M, Wilson VJ, Jones RH, Sonksen PH, Thomas CR. (1992) Intraperitoneal insulin is more potent than subcutaneous insulin at restoring hepatic insulin-like growth factor-I mRNA levels in the diabetic rat: a functional role for the portal vascular link. *J Mol Endocrinol* 1992 Dec;9(3):257-63.

Salgado LR, Semer M, Nery M, Knoepfelmacher M, Lerario AC, Povia G, Jana S, Villares SM, Wajchenberg BL, Liberman B, Nicolau W. (1996) Effect of glycemic control on growth hormone and IGFBP-1 secretion in patients with type I diabetes mellitus. *J Endocrinol Invest*. 1996 Jul-Aug;19(7):433-40.

Santen RJ, Bardin CW. (1973) Episodic luteinizing hormone secretion in man. Pulse analysis, clinical interpretation, physiologic mechanisms. *J Clin Invest*. 1973 Oct;52(10):2617-28.

Savage MO, Dunger DB. (1996) Recombinant IGF-I therapy in insulin-dependent diabetes mellitus. *Diabetes Metab*. 1996 Jul;22(4):257-60.

Schade DS, Eaton RP. (1977) Dose response to insulin in man: differential effects on glucose and ketone body regulation. *J Clin Endocrinol Metab*. 1977 Jun;44(6):1038-53.

Schaefer F, Baumann G, Haffner D, Faunt LM, Johnson ML, Mercado M, Ritz E, Mehls O, Veldhuis JD. (1996) Multifactorial control of the elimination kinetics of unbound (free) growth hormone (GH) in the human: regulation by age, adiposity, renal function, and steady state concentrations of GH in plasma. *J Clin Endocrinol Metab*. 1996 Jan;81(1):22-31

Schaper NC. (1990) Growth hormone in type I diabetic and healthy man. *Acta Endocrinol (Copenh)*. 1990;122 Suppl 2:1-47.

Schaper NC. (1992) Growth hormone and insulin-dependent diabetes mellitus. *Neth J Med*. 1992 Aug;41(1-2):28-35. Review.

Scheen AJ, Lefebvre PJ. (1996) Insulin action in man. *Diabetes Metab*. 1996 Apr;22(2):105-10. Review.

Scheffler WC. (1969) Statistics for biological sciences (1969) Addison-Wesley, Reading Mass.

Schliess F, Haussinger D. (2000) Cell hydration and insulin signalling. *Cell Physiol Biochem*. 2000;10(5-6):403-8.

Schmidt MI, Hadji-Georgopoulos A, Rendell M, Margolis S, Kowarski A. (1981) The dawn phenomenon, an early morning glucose rise: implications for diabetic intraday blood glucose variation. *Diabetes Care*. 1981 Nov-Dec;4(6):579-85.

Schwartz J, Foster CM, Satin MS. (1985) Growth hormone and insulin-like growth factors I and II produce distinct alterations in glucose metabolism in 3T3-F442A adipocytes. *Proc Natl Acad Sci U S A*. 1985 Dec;82(24):8724-8.

Seth J, Hanning I, Bacon RR, Hunter WM. (1988a) Quality of performance of assays for serum growth hormone in the United Kingdom (UK): evidence from the UK external quality assessment scheme, 1980-1987. *Clin Chim Acta*. 1988 May 31;174(2):171-83.

Seth J, Hanning I. (1988b) Factors associated with the quality of laboratory performance in the United Kingdom external quality assessment scheme for serum growth hormone. *Clin Chim Acta*. 1988 May 31;174(2):185-95.

Seta KA, Roth RA. (1997) Overexpression of insulin degrading enzyme: cellular localization and effects on insulin signaling. *Biochem Biophys Res Commun*. 1997 Feb 3;231(1):167-71.

Sheu WH, Hollenbeck CB, Wu MS, Jaspan JB, Chen YD, Reaven GM. (1990) Effect of difference in glucose infusion rate on quantification of hepatic glucose production. *J Clin Endocrinol Metab*. 1990 May;70(5):1354-60.

Shah N, Aloji J, Evans WS, Veldhuis JD. (1999) Time mode of growth hormone (GH) entry into the bloodstream and steady-state plasma GH concentrations, rather than sex, estradiol, or menstrual cycle stage, primarily determine the GH elimination rate in healthy young women and men. *J Clin Endocrinol Metab*. 1999 Aug; 84(8):2862-9.

Sharp PS, Foley K, Vitelli F, Maneschi F, Kohner EM. (1984) Growth hormone response to hyperinsulinaemia in insulin-dependent diabetics. Comparison of patients with and without retinopathy. *Diabet Med*. 1984 May;1(1):55-8.

Sharp PS, Mohan V, Maneschi F, Vitelli F, Cloke HR, Burrin JM, Kohner EM. (1987) Changes in plasma growth hormone in diabetic and nondiabetic subjects during the glucose clamp. *Metabolism*. 1987 Jan;36(1):71-5.

Shaw JH, Wolfe RR. (1984) Influence of beta-hydroxybutyrate infusion on glucose and free fatty acid metabolism in dogs. *Am J Physiol*. 1984 Dec;247(6 Pt 1):E756-64.

Sherwin R, Wahren J, Felig P. (1976a) Evanescent effects of hypo- and hyperglucagonemia on blood glucose homeostasis. *Metabolism*. 1976 Nov;25(11 Suppl 1):1381-3.

Sherwin RS, Hendler RG, Felig P. (1976b) Effect of diabetes mellitus and insulin on the turnover and metabolic response to ketones in man. *Diabetes*. 1976 Sep;25(9):776-84.

Simon C, Brandenberger G, Follenius M. (1988) Absence of the dawn phenomenon in normal subjects. *J Clin Endocrinol Metab*. 1988 Jul;67(1):203-5.

Simonson DC, Tamborlane WV, Sherwin RS, Smith JD, DeFronzo RA. (1985) Improved insulin sensitivity in patients with type I diabetes mellitus after CSII. *Diabetes*. 1985 Aug;34 Suppl 3:80-6.

Sirek OV, Sirek A, Przybylska K, Doolan H, Niki A. (1967) Plasma free fatty acid concentrations in Houssay dogs following a single injection of growth hormone. *Endocrinology*. 1967 Aug;81(2):395-7.

Skamene A, Patel YC. (1984) Infusion of graded concentrations of somatostatin in man: pharmacokinetics and differential inhibitory effects on pituitary and islet hormones. *Clin Endocrinol (Oxf)*. 1984 May;20(5):555-64.

Skor DA, White NH, Thomas L, Shah SD, Cryer PE, Santiago JV. (1983) Examination of the role of the pituitary-adrenocortical axis, counterregulatory hormones, and insulin clearance in variable nocturnal insulin requirements in insulin-dependent diabetes. *Diabetes*. 1983 May;32(5):403-7.

Skutches CL, Owen OE, Reichard GA Jr. (1990) Acetone and acetol inhibition of insulin-stimulated glucose oxidation in adipose tissue and isolated adipocytes. *Diabetes*. 1990 Apr;39(4):450-5.

Smith CP, Dunger DB, Williams AJ, Taylor AM, Perry LA, Gale EA, Preece MA, Savage MO. (1989a) Relationship between insulin, insulin-like growth factor I, and dehydroepiandrosterone sulfate concentrations during childhood, puberty, and adult life. *J Clin Endocrinol Metab*. 1989 May;68(5):932-7.

Smith WC, Kuniyoshi J, Talamantes F. (1989b) Mouse serum growth hormone (GH) binding protein has GH receptor extracellular and substituted transmembrane domains. *Mol Endocrinol*. 1989 Jun;3(6):984-90.

Smith CR, Norman MR. (1990) Prolactin and growth hormone: molecular heterogeneity and measurement in serum. *Ann Clin Biochem*. 1990 Nov;27 (Pt 6):542-50. Review.

Snedecor GW, Cochran WG (1980) Statistical Methods. 7th Edition, Iowa State University Press, Ames, Iowa. Johnson

Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC, Butler PC. (2000) Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. *J Clin Endocrinol Metab.* 2000 Dec;85(12):4491-9.

Sonksen PH, Russell-Jones D, Jones RH. (1993) Growth hormone and diabetes mellitus. A review of sixty-three years of medical research and a glimpse into the future? *Horm Res.* 1993;40(1-3):68-79. Review

Sonksen PH, Srivastava MC, Tompkins CV, Nabarro JD. (1972) Growth-hormone and cortisol responses to insulin infusion in patients with diabetes mellitus. *Lancet.* 1972 Jul 22;2(7769):155-9

Sperling MA, Wollesen F, DeLamater PV. (1973) Daily production and metabolic clearance of growth hormone in juvenile diabetes mellitus. *Diabetologia.* 1973 Oct;9(5):380-3.

Stein DT, Esser V, Stevenson BE, Lane KE, Whiteside JH, Daniels MB, Chen S, McGarry JD. (1996) Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. *J Clin Invest.* 1996 Jun 15;97(12):2728-35.

Stoving RK, Veldhuis JD, Flyvbjerg A, Vinten J, Hangaard J, Koldkjaer OG, Kristiansen J, Hagen C. (1999) Jointly amplified basal and pulsatile growth hormone (GH) secretion and increased process irregularity in women with anorexia nervosa: indirect evidence for disruption of feedback regulation within the GH-insulin-like growth factor I axis. *J Clin Endocrinol Metab.* 1999 Jun;84(6):2056-63.

Suikkari AM, Koivisto VA, Rutanen EM, Yki-Jarvinen H, Karonen SL, Seppala M. (1988) Insulin regulates the serum levels of low molecular weight insulin-like growth factor-binding protein. *J Clin Endocrinol Metab.* 1988 Feb;66(2):266-72.

Svanberg E, Ohlsson C, Kimball SR, Lundholm K. (2000) rhIGF-I/IGFBP-3 complex, but not free rhIGF-I, supports muscle protein biosynthesis in rats during semistarvation. *Eur J Clin Invest.* 2000 May;30(5):438-46.

Svedberg J, Bjorntorp P, Smith U, Lonnroth P. (1990) Free-fatty acid inhibition of insulin binding, degradation, and action in isolated rat hepatocytes. *Diabetes.* 1990 May;39(5):570-4.

Tait JF, Tait SA. (1998) A personal history of the early development of the concept and methods of measurement of the metabolic clearance rate particularly of steroid hormones. *Clin Exp Pharmacol Physiol Suppl.* 1998 Nov;25:S101-18. Review.

- Takahashi K, Ghatei MA, Lam HC, O'Halloran DJ, Bloom SR. (1990) Elevated plasma endothelin in patients with diabetes mellitus. *Diabetologia*. 1990 May;33(5):306-10.
- Takeda S, Podskalny JM, Gorden P. (1984) Direct effect of human growth hormone to inhibit glucose uptake in cultured human fibroblasts. *Metabolism* 1984 Jul;33(7):658-61.
- Tamborlane WV, Sherwin RS, Koivisto V, Hendler R, Genel M, Felig P. (1979) Normalization of the growth hormone and catecholamine response to exercise in juvenile-onset diabetic subjects treated with a portable insulin infusion pump. *Diabetes*. 1979 Aug;28(8):785-8.
- Tannenbaum GS, Rorstad O, Brazeau P. (1979) Effects of prolonged food deprivation on the ultradian growth hormone rhythm and immunoreactive somatostatin tissue levels in the rat. *Endocrinology*. 1979 Jun;104(6):1733-8.
- Tannenbaum GS, Ling N. (1984) The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. *Endocrinology*. 1984 Nov;115(5):1952-7.
- Tannenbaum GS, Bowers CY. (2001) Interactions of growth hormone secretagogues and growth hormone-releasing hormone/somatostatin. *Endocrine*. 2001 Feb;14(1):21-7.
- Taylor AL, Finster JL, Mintz DH. (1969) Metabolic clearance and production rates of human growth hormone. *J Clin Invest*. 1969 Dec;48(12):2349-58.
- Taylor AM, Dunger DB, Preece MA, Holly JM, Smith CP, Wass JA, Patel S, Tate VE. (1990) The growth hormone independent insulin-like growth factor-I binding protein BP-28 is associated with serum insulin-like growth factor-I inhibitory bioactivity in adolescent insulin-dependent diabetics. *Clin Endocrinol (Oxf)*. 1990 Feb;32(2):229-39.
- Taylor AM, Sharma AK, Avasthy N, Duguid IG, Blanchard DS, Thomas PK, Dandona P. (1987) Inhibition of somatomedin-like activity by serum from streptozotocin-diabetic rats: prevention by insulin treatment and correlation with skeletal growth. *Endocrinology* 1987 Oct;121(4):1360-5.
- Thomas MJ. (1998) The molecular basis of growth hormone action. *Growth Horm IGF Res*. 1998 Feb;8(1):3-11. Review.
- Thompson RG, Rodriguez A, Kowarski A, Blizzard RM. (1972) Growth hormone: metabolic clearance rates, integrated concentrations, and production rates in normal adults and the effect of prednisone. *J Clin Invest*. 1972 Dec;51(12):3193-9.

Thorner MO, Hartman ML, Vance ML, Pezzoli SS, Ampleford EJ. (1995) Neuroendocrine regulation of growth hormone secretion. *Neurosci Biobehav Rev.* 1995 Fall;19(3):465-8. Review.

Topper E, Gertner J, Amiel S, Press M, Genel M, Tamborlane WV. (1985) Deranged alpha-adrenergic regulation of growth hormone secretion in poorly controlled diabetes: reversal of the exaggerated response to clonidine after continuous subcutaneous insulin infusion. *Pediatr Res.* 1985 Jun;19(6):534-6.

Torsello A, Panzeri G, Cermenati P, Caroleo MC, Ghigo E, Camanni F, Muller EE, Locatelli V. (1988) Involvement of the somatostatin and cholinergic systems in the mechanism of growth hormone autocrine feedback regulation in the rat. *J Endocrinol.* 1988 May;117(2):273-81.

Trumper BG, Reschke K, Molling J. (1995) Circadian variation of insulin requirement in insulin dependent diabetes mellitus the relationship between circadian change in insulin demand and diurnal patterns of growth hormone, cortisol and glucagon during euglycemia. *Horm Metab Res.* 1995 Mar;27(3):141-7.

Unger JW, Betz M. (1998) Insulin receptors and signal transduction proteins in the hypothalamo-hypophyseal system: a review on morphological findings and functional implications. *Histol Histopathol.* 1998 Oct;13(4):1215-24. Review.

Unger RH, Aguilar-Parada E, Muller WA, Eisentraut AM. (1970) Studies of pancreatic alpha cell function in normal and diabetic subjects. *J Clin Invest.* 1970 Apr;49(4):837-48.

Urban RJ, Kaiser DL, van Cauter E, Johnson ML, Veldhuis JD. (1988) Comparative assessments of objective peak-detection algorithms. II. Studies in men. *Am J Physiol.* 1988 Jan;254(1 Pt 1):E113-9.

Valcavi R, Zini M, Dieguez C, Portioli I, Scanlon MF. (1990) Effect of oral glucose on the late growth hormone rise and growth hormone responses to GHRH in normal subjects. *Clin Endocrinol (Oxf).* 1990 May;32(5):539-43.

Van Cauter E, L'Hermite M, Copinschi G, Refetoff S, Desir D, Robyn C. (1981) Quantitative analysis of spontaneous variations of plasma prolactin in normal man. *Am J Physiol.* 1981 Nov;241(5):E355-63.

Van Cauter E, Kerkhofs M, Caufriez A, Van Onderbergen A, Thorner MO, Copinschi G. (1992) A quantitative estimation of growth hormone secretion in normal man: reproducibility and relation to sleep and time of day. *J Clin Endocrinol Metab.* 1992 Jun;74(6):1441-50.

Van Cauter E, Plat L, Copinschi G. (1998) Interrelations between sleep and the somatotrophic axis. *Sleep.* 1998 Sep 15;21(6):553-66. Review.

Van den Berg G, Veldhuis JD, Frolich M, Roelfsema F. (1996) An amplitude-specific divergence in the pulsatile mode of growth hormone (GH) secretion underlies the gender difference in mean GH concentrations in men and premenopausal women. *J Clin Endocrinol Metab.* 1996 Jul;81(7):2460-7.

Vance ML, Kaiser DL, Evans WS, Furlanetto R, Vale W, Rivier J, Thorner MO. (1985) Pulsatile growth hormone secretion in normal man during a continuous 24-hour infusion of human growth hormone releasing factor (1-40). Evidence for intermittent somatostatin secretion. *J Clin Invest.* 1985 May;75(5):1584-90.

Vance ML, Kaiser DL, Rivier J, Vale W, Thorner MO. (1986) Dual effects of growth hormone (GH)-releasing hormone infusion in normal men: somatotroph desensitization and increase in releasable GH. *J Clin Endocrinol Metab.* 1986 Mar;62(3):591-4.

Vance ML. (1996) Nutrition, body composition, physical activity and growth hormone secretion. *J Pediatr Endocrinol Metab.* 1996 Jun;9 Suppl 3:299-301. Review.

Vanhelder WP, Sirek A, Sirek OV, Norwich KH, Policova Z, Vanhelder T. (1980) Diurnal episodic pattern of insulin secretion in the dog. *Diabetes.* 1980 Apr;29(4):326-8.

Veldhuis JD, Johnson ML. (1986) Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection. *Am J Physiol.* 1986 Apr;250(4 Pt 1):E486-93.

Veldhuis JD, Carlson ML, Johnson ML. (1987) The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. *Proc Natl Acad Sci U S A.* 1987 Nov;84(21):7686-90.

Veldhuis JD, Johnson ML (1988a) A novel general biophysical model for simulating episodic endocrine gland signaling. *Am J Physiol.* 1988 Dec;255(6 Pt 1):E749-59.

Veldhuis JD, Johnson ML. (1988b) In vivo dynamics of luteinizing hormone secretion and clearance in man: assessment by deconvolution mechanics. *J Clin Endocrinol Metab.* 1988 Jun;66(6):1291-300.

Veldhuis JD, Johnson ML. (1990) Returning to the roots of endocrinology: the challenge of evaluating in vivo glandular secretory activity. *Endocrinology.* 1990 Dec;127(6):2611-7.

Veldhuis JD, Johnson ML, Lizarralde G, Iranmanesh A. (1992) Rhythmic and nonrhythmic modes of anterior pituitary gland secretion. *Chronobiol Int.* 1992 Oct;9(5):371-9.

Veldhuis JD, Johnson ML, Faunt LM, Mercado M, Baumann G. (1993)

Influence of the high-affinity growth hormone (GH)-binding protein on plasma profiles of free and bound GH and on the apparent half-life of GH. Modeling analysis and clinical applications. *J Clin Invest.* 1993 Feb;91(2):629-41.

Veldhuis JD, Liem AY, South S, Weltman A, Weltman J, Clemmons DA, Abbott R, Mulligan T, Johnson ML, Pincus S, et al. (1995)

Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. *J Clin Endocrinol Metab.* 1995 Nov;80(11):3209-22.

Veldhuis JD. (1996a) Gender differences in secretory activity of the human somatotrophic (growth hormone) axis. *Eur J Endocrinol.* 1996 Mar;134(3):287-95. Review

Veldhuis JD. (1996b) New modalities for understanding dynamic regulation of the somatotrophic (GH) axis: explication of gender differences in GH neuroregulation in the human. *J Pediatr Endocrinol Metab.* 1996 Jun;9 Suppl 3:237-53.

Veldhuis JD. (1996c) What have we learned so far from ultrasensitive growth hormone (GH) assays? *Clin Chem.* 1996 Nov;42(11):1731-2.

Veldhuis JD. (1998) Neuroendocrine control of pulsatile growth hormone release in the human: relationship with gender. *Growth Horm IGF Res.* 1998 Apr;8 Suppl B:49-59.

Veldhuis JD, Roemmich JN, Rogol AD. (2000) Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females—a general clinical research center-based study. *J Clin Endocrinol Metab.* 2000 Jul;85(7):2385-94.

Veldhuis JD, Anderson SM, Shah N, Bray M, Vick T, Gentili A, Mulligan T, Johnson ML, Weltman A, Evans WS, Iranmanesh A. (2001)

Neurophysiological regulation and target-tissue impact of the pulsatile mode of growth hormone secretion in the human. *Growth Horm IGF Res.* 2001 Jun;11 Suppl A:S25-37. Review.

Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J. (1994) Effect of hyperketonemia and hyperlacticacidemia on symptoms, cognitive dysfunction, and counterregulatory hormone responses during hypoglycemia in normal humans. *Diabetes.* 1994 Nov;43(11):1311-7.

Verdin E, Castillo M, Luyckx AS, Lefebvre PJ. (1984) Similar metabolic effects of pulsatile versus continuous human insulin delivery during euglycemic, hyperinsulinemic glucose clamp in normal man. *Diabetes.* 1984 Dec;33(12):1169-74.

- Vigneri R, Squatrito S, Pezzino V, Filetti S, Branca S, Polosa P. (1976) Growth hormone levels in diabetes. Correlation with the clinical control of the disease. *Diabetes*. 1976 Mar;25(3):167-72.
- Villas-Boas Weffort RF, Ramos-Dias JC, Chipoch C, Lengyel AM. (1997) Growth hormone (GH) response to GH-releasing peptide-6 in patients with insulin-dependent diabetes mellitus. *Metabolism*. 1997 Jun;46(6):706-10.
- Wagner C, Caplan SR, Tannenbaum GS. (1998) Genesis of the ultradian rhythm of GH secretion: a new model unifying experimental observations in rats. *Am J Physiol*. 1998 Dec;275(6 Pt 1):E1046-54.
- Wahba ZZ, Soliman KF. (1988) Effect of diabetes on the enzymes of the cholinergic system of the rat brain. *Experientia*. 1988 Sep 15;44(9):742-6.
- Waldhausl WK, Bratusch-Marrain P, Kruse V, Jensen I, Nowotny P, Vierhapper H. (1985) Effect of insulin antibodies on insulin pharmacokinetics and glucose utilization in insulin-dependent diabetic patients. *Diabetes*. 1985 Feb;34(2):166-73.
- Waldhausl WK, Bratusch-Marrain PR, Francesconi M, Nowotny P, Kiss A. (1982) Insulin production rate in normal man as an estimate for calibration of continuous intravenous insulin infusion in insulin-dependent diabetic patients. *Diabetes Care*. 1982 Jan-Feb;5(1):18-24.
- Walker M, Fulcher GR, Sum CF, Orskov H, Alberti KG. (1991) Effect of glycemia and nonesterified fatty acids on forearm glucose uptake in normal humans. *Am J Physiol*. 1991 Sep;261(3 Pt 1):E304-11.
- Walters JM, Ward GM, Kalfas A, Best JD, Alford FP. (1992) The effect of epinephrine on glucose-mediated and insulin-mediated glucose disposal in insulin-dependent diabetes. *Metabolism*. 1992 Jun;41(6):671-7.
- Wass JA. (1983) Growth hormone neuroregulation and the clinical relevance of somatostatin. *Clin Endocrinol Metab*. 1983 Nov;12(3):695-724. Review
- Weiss J, Cronin MJ, Thorner MO. (1987) Periodic interactions of GH-releasing factor and somatostatin can augment GH release in vitro. *Am J Physiol*. 1987 Nov;253(5 Pt 1):E508-14.
- Welbourne TC, Milford L, Carter P. (1997) The role of growth hormone in substrate utilization. *Baillieres Clin Endocrinol Metab* 1997 Dec;11(4):699-707
- West TE, Sonksen PH. (1977) Is the growth-hormone response to insulin due to hypoglycaemia, hyperinsulinaemia or a fall in plasma free fatty acids? *Clin Endocrinol (Oxf)*. 1977 Oct;7(4):283-8.
- West IC. (2000) Radicals and oxidative stress in diabetes. *Diabet Med*. 2000 Mar;17(3):171-80. Review

Whincup G, Milner RD. (1987) Prediction and management of nocturnal hypoglycaemia in diabetes. *Arch Dis Child.* 1987 Apr;62(4):333-7.

Widmer A, Keller U, Pasquel M, Berger W. (1988) Alterations in insulin clearance and hepatic blood flow during the night do not contribute to the 'dawn phenomenon' in type 1 diabetes. *Horm Res.* 1988;29(5-6):197-201.

Wildenhoff KE. (1972) Diurnal variations in the concentrations of blood acetoacetate, 3-hydroxybutyrate and glucose in normal persons. *Acta Med Scand.* 1972 Apr;191(4):303-6.

Wildenhoff KE, Johansen JP, Karstoft H, Yde H, Sorensen NS. (1974) Diurnal variations in the concentrations of blood acetoacetate and 3-hydroxybutyrate. The ketone body peak around midnight and its relationship to free fatty acids, glycerol, insulin, growth hormone and glucose in serum and plasma. *Acta Med Scand.* 1974 Jan-Feb;1-2(1):25-8.

Williamson JR, Corkey BE. (1979) Assay of citric acid cycle intermediates and related compounds--update with tissue metabolite levels and intracellular distribution. *Methods Enzymol.* 1979;55:200-22.

Wilson DB, Wyatt DP. (1988) Immunofluorescent analysis of somatotroph distribution in the adenohypophysis of developing lit/lit mice. *J Anat.* 1988 Feb;156:51-9.

Winer LM, Shaw MA, Baumann G. (1990) Basal plasma growth hormone levels in man: new evidence for rhythmicity of growth hormone secretion. *J Clin Endocrinol Metab.* 1990 Jun;70(6):1678-86.

Wurzbarger MI, Prelevic GM, Sonksen PH, Peric LA, Till S, Morris RW. (1990) Effects of improved blood glucose on insulin-induced hypoglycaemia, TRH, GnRH and exercise tests in insulin-dependent diabetes. *Clin Endocrinol (Oxf).* 1990 Jun;32(6):799-807.

Wurzbarger MI, Sonksen PH. (1996) Natural course of growth hormone hypersecretion in insulin-dependent diabetes mellitus. *Med Hypotheses.* 1996 Feb;46(2):145-9. Review.

Yamashita S, Melmed S. (1986) Effects of insulin on rat anterior pituitary cells. Inhibition of growth hormone secretion and mRNA levels. *Diabetes.* 1986 Apr;35(4):440-7.

Yamashita S, Melmed S. (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology.* 1986 Jan;118(1):176-82.

Yki-Jarvinen H, Koivisto VA. (1983) Effects of body composition on insulin sensitivity. *Diabetes.* 1983 Oct;32(10):965-9.

Yki-Jarvinen H, Koivisto VA. (1986) Natural course of insulin resistance in type I diabetes. *N Engl J Med.* 1986 Jul 24;315(4):224-30.

Zadik Z, Chalew SA, McCarter RJ Jr, Meistas M, Kowarski AA. (1984) The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. *J Clin Endocrinol Metab.* 1985 Mar;60(3):513-6.

Zadik Z, Kayne R, Kappy M, Plotnick LP, Kowarski AA. (1980) Increased integrated concentration of norepinephrine, epinephrine, aldosterone, and growth hormone in patients with uncontrolled juvenile diabetes mellitus. *Diabetes.* 1980 Aug;29(8):655-8.

BIBLIOGRAPHY

List of Publications and Presentations arising from Thesis

Pal BR, Mullis PE, Matthews DR, Hindmarsh PC, Dunger DB

Half-life of Exogenous Growth Hormone (GH) following suppression of Endogenous GH secretion with somatostatin in Type I (insulin-dependent) Diabetes Mellitus *Clinical Endocrinology* 36(3):255-63, 1992 Mar

Pal BR, Matthews DR, Mullis PE, Edge JA, Hindmarsh PC, Dunger DB

The frequency and amplitude of Growth hormone secretory episodes as determined by deconvolution analysis are increased in adolescents with insulin dependent diabetes mellitus and are unaffected by short-term euglycaemia *Clinical Endocrinology* 38(1):93-100, 1993 Jan

Pal BR, Phillips PE, Matthews DR, Dunger DB

Contrasting metabolic effects of continuous and pulsatile growth hormone administration in young adults with Insulin Dependent Diabetes Mellitus *Diabetologia* 35(6):542-9, 1992 Jun

Dunger DB, Edge JA, Pal R, Taylor AM, Holly JM, Matthews DR

The impact of increased Growth hormone secretion on carbohydrate metabolism in adolescents with diabetes
Acta Paediatrica Scandinavica - Supplement 377:69-77; discussion 78, 1991

ESPE 29th Annual meeting, Vienna (Sept 1990)

The half-life of Exogenous Growth Hormone after suppression of Endogenous Growth Hormone in Type I Diabetes Mellitus

British Paediatric Association 63rd Annual meeting, Warwick (April 1991)

An analysis of the half-life, volume of distribution and secretion of Growth Hormone in adolescents with diabetes

British Endocrine Society, Brighton (April 1991)

The half-life, volume of distribution and secretory rates of growth hormone in adolescents with insulin dependent diabetes mellitus

ESPE 30th Annual meeting, Berlin (August 1991)

Effects of Euglycaemia and Pirenzepine on the secretory dynamics of Growth hormone (GH) as predicted by Deconvolution analysis in adolescents with IDDM

ESPE 30th Annual meeting, Berlin (August 1991)

Effects of continuous versus pulsatile overnight growth hormone (GH) in young adults with diabetes

ESPE 33rd Annual meeting, Maastricht, The Netherlands (June 1994)

GH suppression with somatostatin is antagonised by hyperinsulinaemia in young adults with Type 1 diabetes

Statistical References

Altman DG, *Practical Statistics for Medical Research*, Chapman & Hall/CRC Statistics and Mathematics; ISBN: 0412276305

Altman D & Machin D, *Statistics with confidence*, BMJ Books; ISBN: 0727913751

Armitage P, Matthews JNS, Berry G, *Statistical Methods in Medical Research*, Blackwell Science (UK); ISBN: 0632052570

Bland M, *An Introduction to Medical Statistics*, Oxford University Press; ISBN: 0192632698

Chatfield C, *The Analysis of Time Series*, Chapman & Hall, CRC Statistics and Mathematics; ISBN: 0412716402

Coakes SJ, Steed LG, *SPSS for Windows*, Jacaranda Wiley; ISBN: 0471340804

Conover WJ, *Practical Nonparametric Statistics*, John Wiley and Sons (WIE); ISBN: 0471160687

Meyer R, Krueger D, *A Minitab Guide to Statistics*, Prentice Hall; ISBN: 0130141569

Motulsky H, *Intuitive Biostatistics*, Oxford University Press INC, USA; ISBN: 0195086074

Petrie, *Medical Statistics at a Glance*, Blackwell Science (UK); ISBN: 0632050756

Shumway R, *Time Series Analysis and Its Applications*, Springer-Verlag New York Inc; ISBN: 0387989501

'Dedicated to my father, Mr Prafulla Pal and mother Mrs Anita Pal'

**THE PAST IS OUR FOUNDATION, THE FUTURE IS OUR HOPE OF
INSPIRATION AND FOR THE PRESENT WE LIVE OUR BEST EXISTENCE'**